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# *The Journal of Laboratory and Clinical Medicine*

VOL. VIII

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No. 1

## *ORIGINAL ARTICLES*

### TEMPORARY DISTURBANCES DUE TO LOCAL ANESTHETICS\*

BY ELLISON L. ROSS, PH.D., M.D., CHICAGO, ILL.

TEMPORARY disturbances to the system, usually slight, but sometimes of a severe character, may be expected following the use of local anesthetics. These disturbances, chiefly in the circulatory system, are of considerable concern to both physicians and dentists.

The drugs used locally are cocain and adrenalin or novocain and adrenalin, often accompanied by morphine and atropine. The action of each of these drugs when injected into the circulation has been thoroughly worked out by many investigators. The action of two of the drugs used in succession, either applied with a sponge upon or injected under the mucous membrane, has not been so fully investigated. Adrenalin or pituitrin, for instance, do not have the same effect given by way of the stomach that they have when administered hypodermically. It was therefore considered best for experimental purposes to administer the drugs exactly as they are used in medical practice, so that the results might be comparable.

The object of this work, then, was to determine changes taking place in the respiratory and circulatory systems when the commonly used local anesthetics were applied to animals by the same method used in medical practice.

#### EXPERIMENTAL WORK

Dogs were used as subjects. The work was done on the animals under general anesthesia induced by the intraperitoneal injection of 1. c.c. of 25 per cent of chloretone in almond oil, per kilogram of body weight. Kymographic records were taken of arterial pressure from the carotid and the intracranial venous pressure from the Torcular Herophili. The respiratory

\*From the Department of Physiology and Pharmacology, Northwestern University Medical School. Received for publication, April 17, 1922.

TABLE I  
EFFECTS OF SUBCUTANEOUS INJECTIONS OF ADRENALIN AND ADRENALIN-COCAIN

DOG	ARTERIAL PRESSURE			VENOUS PRESSURE			HEART RATE			HEART AMPLITUDE			RESPIRATION RATE		
	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN
1	52	76	170	.5	.8	-	174	171	156	8	8	11	-	-	-
2	42	42	150	.2	.15	1.1	144	150	96	7	5	20	-	-	48
3	70	72	190	1.9	2.0	6.2	240	222	120	5	5	25	21	24	72
4	70	70	230	.9	.9	4.2	162	162	144	6	6	12	54	66	72
5	66	66	230	.9	.9	8.9	126	126	126	13	13	45	21	21	72
Average	60	65	194	.9	.9	5.1	169	167	128	8	7	23	32	37	64

TABLE II  
EFFECTS OF SUBCUTANEOUS INJECTIONS OF ADRENALIN AND ADRENALIN-COCAIN AFTER MORPHINE-ATROPINE

DOG	ARTERIAL PRESSURE			VENOUS PRESSURE			HEART RATE			HEART AMPLITUDE			RESPIRATION RATE		
	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN	BEFORE	AFTER ADREN.	AFTER ADREN.-COCAIN
1	46	46	184	2.5	2.5	3.2	138	156	144	10	10	12	22	34	32
2	41	48	180	.6	.8	4.8	126	138	216	10	9	2	24	20	22
3	78	78	180	.6	.6	7.8	186	180	120	3	2	10	4	20	36
4	74	64	190	.9	.8	1.8	210	216	180	3	4	3	46	23	36
5	50	48	140	1.1	1.1	3.2	162	129	54	13	12	20	28	30	10
Average	58	57	175	1.1	1.2	4.2	152	162	143	8	7	9	25	25	25

rate was obtained from the tracing of arterial pressure. The arterial and venous pressures were measured in millimeters of mercury, and the amplitude of heart beat in millimeters on the tracing. The rate of pulse and respirations per minute were determined.

The venous pressure was taken with the aid of a manometer filled with sodium carbonate solution of a specific gravity just one-tenth that of mercury.

The first group of dogs was given an injection of  $33\frac{1}{3}$  per cent of the 1:1000 adrenalin, 0.15 c.c. per kilogram of animal. This amount was injected into three or four places in the tonsillar region. After five minutes an injection was made in the same region of a mixture of cocain and adrenalin. This mixture consisted of  $33\frac{1}{3}$  per cent of adrenalin (1:1000) and  $13\frac{1}{3}$  per cent cocain. The arterial pressure, venous pressure, rate and amplitude of heart beat and respiratory rate were estimated for the five animals. The results are given in Table I.

The second group of animals was treated in the same way except that half an hour before the adrenalin was injected the animals were given subcutaneously  $\frac{1}{8}$  c.c. of a 4 per cent morphine sulphate and  $\frac{1}{10}$  c.c. of  $\frac{1}{10}$  per cent atropine sulphate per kilogram of body weight. The results are given in Table II.

TABLE III  
EFFECTS OF SUBMUCOUS INJECTIONS OF ADRENALIN AND COCAIN

DOG	ARTERIAL PRESSURE		VENOUS PRESSURE		HEART RATE		HEART AMPLITUDE		RESPIRATION RATE	
	BEFORE COCAIN	AFTER COCAIN	BEFORE COCAIN	AFTER COCAIN	BEFORE COCAIN	AFTER COCAIN	BEFORE COCAIN	AFTER COCAIN	BEFORE COCAIN	AFTER COCAIN
1	66	165	0	1.1	198	180	8	4	64	54
2	80	110	1.1	1.2	174	182	3	3	26	42
3	84	208	1.0	2.2	138	162	5	6	24	50
4	86	150	2.6	4.8	132	144	9	6	56	52
5	94	116	1.8	3.7	144	144	5	6	56	48
Average	82	150	1.3	2.6	157	162	6	5	45	49

The third group was given the same dose of adrenalin in the usual manner and five minutes later, 0.15 c.c. per kilogram of weight, a solution containing  $13\frac{1}{3}$  per cent cocain was injected into the tonsillar region. The results are given in Table III. The observations were made just before the cocain was injected and at the height of its action.

The fourth, fifth and sixth groups show the effect that swabbing the throat with cocain had on the reaction to the injections of adrenalin.

The fourth group of animals was given 0.15 c.c. of 50 per cent adrenalin (1:1000) per kilogram of weight. After all effects had passed off the throat was swabbed vigorously with 20 per cent cocain. Within 2 minutes the same dose of adrenalin as that given previously was injected into the tonsillar region. The results are given in Table IV.

The animals of the fifth group were given a mixture containing 50 per cent adrenalin (1:1000) and 20 per cent novocain. This was injected into the tonsillar region—0.15 c.c. per kilogram of the animal's weight. After the

TABLE IV  
EFFECTS OF SUBMUCOUS INJECTIONS OF ADRENALIN PRECEDED BY SWABBING THROAT WITH COCAIN

DOG	ARTERIAL PRESSURE			VENOUS PRESSURE			HEART RATE			HEART AMPLITUDE			RESPIRATION RATE		
	NORMAL	ADREN- ALIN	ADREN. AFTER COCAIN	NORMAL	ADREN- ALIN	ADREN. AFTER COCAIN	NORMAL	ADREN- ALIN	ADREN. AFTER COCAIN	NORMAL	ADREN- ALIN	ADREN. AFTER COCAIN	NORMAL	ADREN- ALIN	ADREN. AFTER COCAIN
1	94	84	234	2.0	1.2	3.8	138	126	102	6	6	12	34	30	18
2	70	90	230	.9	1.0	5.2	150	150	162	10	9	7	10	36	41
3	72	78	160	1.6	1.7	4.6	210	186	204	7	7	10	50	50	100
4	100	80	190	-	-	-	150	180	168	7	7	14	10	21	40
5	80	82	170	1.6	1.8	4.2	168	174	180	4	4	15	39	48	24
Average	83	83	197	1.5	1.4	4.4	163	163	163	7	7	12	27	38	45

TABLE V  
EFFECTS OF SUBMUCOUS INJECTIONS OF NOVOCAIN AND ADRENALIN PRECEDED BY SWABBING THROAT WITH COCAIN

DOG	ARTERIAL PRESSURE			VENOUS PRESSURE			HEART RATE			HEART AMPLITUDE			RESPIRATION RATE		
	NORMAL	NOVO- ADREN.	NOVO- ADREN. AFTER COCAIN	NORMAL	NOVO- ADREN.	NOVO- ADREN. AFTER COCAIN	NORMAL	NOVO- ADREN.	NOVO- ADREN. AFTER COCAIN	NORMAL	NOVO- ADREN.	NOVO- ADREN. AFTER COCAIN	NORMAL	NOVO- ADREN.	NOVO- ADREN. AFTER COCAIN
1	78	118	188	1.1	1.3	2.6	156	180	192	5	4	4	36	36	76
2	50	116	140	1.1	1.6	2.1	180	144	120	5	6	7	34	34	42
3	46	36	172	2.2	2.0	9.2	138	138	180	8	6	2	44	58	32
4	70	94	138	.6	.9	1.5	150	210	210	8	7	3	40	18	24
5	112	100	190	1.8	1.6	2.5	150	168	120	3	3	12	22	12	62
Average	71	93	166	1.4	1.5	3.6	155	168	164	6	5	5	38	32	47



action of this injection had disappeared the throat was swabbed with 20 per cent cocain. Within two minutes the mixture of novocain and adrenalin was again injected. The results are given in Table V.

The sixth group was given 0.15 c.c. of 50 per cent of adrenalin (1:1000) per kilogram of weight. When the effect of the adrenalin's action had disappeared the throat was swabbed with 20 per cent cocain. Observations were made just before the cocain was applied and at the height of its action. The results are arranged in Table VI.

#### DISCUSSION

The first problem was the determination of the proper submucous dosage of adrenalin for comparative purposes. This included not only the amount of the adrenalin, but the concentration of the preparation. After much time had been consumed experimenting on a considerable number of animals the dosage of 0.15 c.c. of  $33\frac{1}{3}$  per cent adrenalin (1:1000) per kilogram of the animal's weight was decided upon. This dose was active to the minimum and thus allowed a maximum range of action for any drug used with it.

The submucous dose of cocain used was 0.15 c.c. of  $13\frac{1}{3}$  per cent solution of cocain hydrochloride per kilogram of body weight.

The first experiment was designated to demonstrate whether any considerable reaction takes place when minimal doses of adrenalin and cocain are injected together. Adrenalin alone changed the average arterial pressure from 60 to 65 mm., venous pressure from 0.9 to 0.9 mm., rate of heart beat from 169 to 167 beats per minute, force of heart beat from 8 to 7 and respiratory rate from 32 to 37 per minute. Expressed in per cent, adrenalin produced an increase in arterial pressure of 8, in respiratory rate of 16 and a decrease in rate of heart beat of 1, and in amplitude of heart beat of 13. Venous pressure was not changed by this dosage of adrenalin. In general it may be stated that the effect of this dose of adrenalin is minimal.

This same series of animals was given submucously per kilogram of weight 0.15 c.c. of a solution containing adrenalin  $33\frac{1}{3}$  per cent and cocain hydrochloride  $13\frac{1}{3}$  per cent. This solution was given after the reaction of the adrenalin alone had completely disappeared. The maximum action of this mixture produced an average of 194 mm. of arterial pressure, 5.1 mm. of venous pressure, a rate of heart beat of 128 per minute, a heart amplitude of 23 mm. and a respiratory rate of 54 per minute. Expressed in percentage of the normal there was a great increase in all but one of the factors measured. The rate of heart beat was decreased 18 per cent. There was an increase of 233 per cent in arterial pressure, 467 per cent in venous pressure, 187 per cent in amplitude of heart beat and 100 per cent in respiratory rate. In general, it may be stated that the reaction was enormous except in the rate of the heart beat.

It is very significant that the increase of arterial pressure was only about half of the increase of the intracranial venous pressure. This condition would produce cerebral asphyxia, which might induce all of the temporary disturbances occurring in the use of local anesthetics in medical practice.

Cerebral asphyxia causes a feeling of smothering, accompanied by some excitement and followed by faintness and dimness of vision.

It is a common practice to give hypodermic injections of morphine and atropine a short period before an operation which is to be done under local anesthesia. It was a question whether such premedication would increase or decrease the disturbances produced by cocain and adrenalin. The principal influence that morphine might exert on the factors under consideration is a retardation of the heart by vagus stimulation and also a retardation of

TABLE VI  
EFFECTS OF SWABBING THROAT WITH COCAIN

DOG	ARTERIAL PRESSURE		VENOUS PRESSURE		HEART RATE		HEART AMPLITUDE		RESPIRATION RATE	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
1	164	210	2.5	5.2	168	156	6	13	24	44
2	130	130	.9	1.0	138	144	5	8	66	60
3	88	94	.8	.7	114	138	9	7	34	64
4	88	86	.5	.5	144	150	9	8	52	48
5	108	152	1.0	1.3	144	144	12	7	26	38
Average	116	134	1.1	1.7	142	146	8	9	40	51

TABLE VII  
TABLE OF AVERAGES

EXPERIMENT		ARTERIAL PRESSURE	VENOUS PRESSURE	HEART RATE	HEART AMPLITUDE	RESPIRATION RATE
1	Normal	60	.9	169	8	32
1	Adrenalin After	65	.9	167	7	37
1	Adrenalin and Cocain After	194	5.1	128	23	64
2	Morphine-Atropine K 1/2 Hour Preceding	58	1.1	152	8	25
2	Adrenalin After	57	1.2	162	7	25
2	Adrenalin and Cocain After	175	4.2	143	9	25
3	Cocain—Long After Adren.	82	1.3	157	6	45
3	Cocain After	150	2.6	162	5	49
4	Normal	83	1.5	163	7	27
4	Adrenalin After	83	1.4	163	7	38
4	Adrenalin After Cocain Swab After	197	4.4	163	12	45
5	Normal	71	1.4	155	6	38
5	Adrenalin—Novocain After	93	1.5	168	5	32
5	Adren.—Novocain After Cocain S. After	166	3.6	164	5	47
6	Before Cocain Swab—After Adrenalin	116	1.1	142	8	40
6	After Cocain Swab	134	1.7	146	9	51

the respiration by depression of the respiratory center. Atropine would exert its chief influence to increase the rate of heart beat through paralysis of the vagus nerve endings. This action of atropine would tend to counter-balance the action of morphine on the rate of the heart beat.

These conclusions reached through deduction could only be proved or disproved by making the actual tests. Consequently a group of animals was given hypodermic injections of morphine and atropine sulphate in doses comparable with those used in medical practice. The injections were made

half an hour before the submucous injections were begun. The same drugs in the same doses were used as with the preceding set of animals.

The increases of 202 and 282 per cent in the arterial and venous pressures are not as great as those noticed in animals without the morphine and atropine. The decrease of the rate of heart beat was only 6 per cent, while without premedication it was 18 per cent. The most striking difference was in the absence of any change in either the amplitude of heart beat or respiratory rate. This constancy was probably due to the action of atropine. The effect of the morphine, depressing the respiratory center, seems to have been just sufficient to counterbalance the stimulation through asphyxia of the respiratory center produced by the adrenalin and cocain. A certain amount of the reduction of the reactions to cocain and adrenalin observed in the premedication with morphine and atropine must be due to the increased depth of general anesthesia produced by the chloretone and morphine.

In view of these various phases of action it must be concluded that premedication with morphine and atropine does not reduce the main factors involved in the temporary disturbances from injections of cocain and adrenalin. It may be significant that from the considerable number of animals that had been given the cocain only two died and these two had both been treated with morphine and atropine.

A third group of animals was used in the attempt to explain the cause of the great changes in blood pressure, action of the heart and respiration produced by injections of adrenalin and cocain. Adrenalin administered alone in the quantity and manner employed was not the cause of the changes, as is shown by the results obtained from the first group of dogs. The third group was used to estimate the effect of cocain alone. Adrenalin was given before the cocain in order that the comparison might be made with the first group. The cocain increased arterial pressure 83 per cent, venous pressure 100 per cent, rate of the heart beat 3 per cent and rate of respiration 9 per cent but decreased the amplitude of heart beat 13 per cent. It is very clear that the blood pressure was altered two to four times as much by the combined action of adrenalin and cocain as by the individual action of either. This synergistic action of cocain and adrenalin is well known to occur when adrenalin is injected intravenously after intravenous injection of cocain. That marked synergy is present when adrenalin and cocain are injected submucously has not been demonstrated.

Since cocain is capable of so great an influence over the reaction to adrenalin, it was thought interesting to determine whether such small amounts of cocain as are absorbed when the pharynx is swabbed with 20 per cent cocain were sufficient to increase the sensitiveness to adrenalin.

A submucous injection of 0.15 c.c. of a 50 per cent adrenalin (1:1000) was used as the dosage. After this had been given time to disappear 20 per cent cocain was swabbed well on the pharynx and shortly after another similar dose of adrenalin was injected. The first injections of adrenalin produced no change in any of the factors except a decrease of 7 per cent in venous pressure and an increase of 41 per cent in rate of respiration. The

second injection of adrenalin which followed the swabbing of cocain produced an increase of 13 per cent in arterial pressure, 193 per cent in venous pressure, 71 per cent in amplitude of heart beat, and 67 per cent in rate of respiration. No change was produced in rate of heart beat.

Novocain is one of the most commonly used local anesthetics. That novocain with adrenalin does not eliminate the strong synergistic action of cocain on the reaction to adrenalin is shown by the results obtained with the fifth group of animals.

The preceding method was followed except that the solution of adrenalin contained also 20 per cent of novocain. The first injection of novocain and adrenalin produced an increase of 31 per cent in arterial pressure, 7 per cent

TABLE VIII  
TABLE OF AVERAGES—EXPRESSED IN PER CENT OF THE NORMAL

EXPERIMENT		ARTERIAL PRESSURE	VENOUS PRESSURE	HEART RATE	HEART AMPLITUDE	RESPIRATION RATE
1	Normal	100	100	100	100	100
1	Adrenalin After	108	100	99	87	116
1	Adrenalin and Cocain After	323	567	82	287	200
2	Morphine and Atropine (½ hr. preceding)	100	100	100	100	100
2	Adrenalin After	98	109	107	87	100
2	Adrenalin and Cocain After	302	382	94	112	100
3	Normal—Long After Adrenalin	100	100	100	100	100
3	Cocain After	183	200	103	83	109
4	Normal	100	100	100	100	100
4	Adrenalin After	100	93	100	100	141
4	Adrenalin After Cocain Swab	237	293	100	100	167
5	Normal	100	100	100	100	100
5	Adrenalin and Novocain	131	107	108	83	84
5	Adrenalin and Novocain After Cocain S.	234	257	106	83	124
6	Before Cocain Swab—After Adrenalin	100	100	100	100	100
6	After Cocain Swab—After Adrenalin	116	155	103	112	127

in venous pressure, 8 per cent in rate of heart beat and a decrease of 17 per cent in amplitude of heart beat and 16 per cent in rate of respiration.

The second injection of novocain and adrenalin produced an increase of 134 per cent in arterial pressure, 128 per cent in venous pressure, 6 per cent in rate of heart beat and 24 per cent in respiratory rate. The amplitude of heart beat decreased 17 per cent. Novocain clearly does not interfere with the synergistic action of cocain and adrenalin.

To determine what changes would be accomplished by swabbing 20 per cent cocain on the pharynx a series of animals was taken. These animals were given the same dosage of adrenalin as the fifth group and ten minutes later 20 per cent cocain was swabbed on the pharynx. This swabbing increased the arterial pressure 20 per cent, the venous pressure 55 per cent, the rate of heart beat 3 per cent, amplitude of heart beat 12 per cent and the rate of respiration 12 per cent. Most of these average increases were due to the rather marked reaction of one animal. The other four were very little



affected. These average increases were relatively small compared with the increases due to the swabbing of cocain and the injection of adrenalin.

Therefore we must conclude that swabbing the throat with 20 per cent cocain sensitizes an animal to adrenalin to a marked degree. Lewis and Froelich<sup>1</sup> first demonstrated a synergy between cocain and adrenalin. By intravenous injections of these drugs they showed that adrenalin produced a much greater rise in arterial pressure after the cocain had been administered.

Tatum<sup>2</sup> from a number of observations on the response of the sympathetic nervous system to various stimuli, concludes that cocain increases the responsivity of the peripheral neuromuscular mechanism of the sympathetic system.

In the Report of the Committee on Local Anesthetics in Ophthalmic Work<sup>3</sup> the following statement is made: "Some observers are inclined to believe that the mild toxic symptoms seen after instillations of solutions of cocain may be due to psychic influences as much as to the toxic effects of the cocain." The results presented seem to bear out the contrary conclusions.

Adrenalin is always used with cocain. If we accept the common view that excitement increases the secretion of adrenalin the reaction may be very much increased by the presence of cocain at the time of the excitement.

#### SUMMARY AND CONCLUSIONS

A group of animals was given submucous injections of a minimally active dose of adrenalin, and five minutes later a submucous injection of adrenalin with cocain of the same amount and same concentration. The arterial pressure, venous pressure, heart beat, heart amplitude and respiratory rate were recorded.

A second group of animals was treated in the same way. One hour later a moderate dose of morphine and atropine was given hypodermically.

A third group of animals was given adrenalin in the same manner, and with the same dosage as in Group 1, followed by the usual dose of cocain.

A fourth group of animals was given submucous injections of adrenalin, followed by swabbing the pharynx with 20 per cent of cocain and a repetition of the submucous dose of adrenalin.

A fifth group of animals was given submucous injections of adrenalin and 20 per cent novocain, followed by swabbing of the pharynx with 20 per cent cocain and a repetition of the injection of adrenalin and novocain.

A sixth group of animals was given submucous injections of adrenalin, followed by swabbing of the throat with 20 per cent cocain.

The results of these procedures lead us to the following conclusions:

1. Submucous injections of adrenalin and cocain bring on systemic reactions similar to those produced by intravenous injections of the drug.

2. Submucous injections of cocain and adrenalin are capable of causing enormous increases in arterial and venous pressures. The intracranial venous pressure was increased relatively twice as much as the arterial pressure.

3. Cocain and adrenalin, when injected submucously, act strongly synergistically.

4. Sufficient cocain is absorbed from sponging the pharynx with 20 per cent cocain to increase markedly the sensitiveness of the system to the action of adrenalin.

5. Novocain in a concentration of 20 per cent does not neutralize to any great extent the synergistic action of cocain and adrenalin.

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<sup>2</sup>Tatum, A. L.: Jour. Pharmacol. and Exper. Med., xvi, 109.

<sup>3</sup>Report Committee Local Anesthetics in Ophthalmic Work, Jour. Am. Med. Assn., lxxvii, 1730.

## RETICULOSIS—INCREASED PERCENTAGE OF RETICULATED ERYTHROCYTES IN THE PERIPHERAL BLOOD\*

BY EDWARD B. KRUMBHAAR, M.D., PH.D., PHILADELPHIA, PA.

THE presence of reticulated or "skeined" erythrocytes (hematies granulæuses) in the peripheral blood (as demonstrated by vital staining) has excited interest for many years, and in the last decade has assumed clinical importance as an index of the activity of blood formation. I would suggest as a matter of convenience that when the normal percentage of these cells in the peripheral blood is exceeded, the condition be designated "reticulosis," to replace some such expression as "an increase in the number of reticulated cells," as is now the custom. The word "reticulocyte" might similarly be substituted for "reticulated erythrocytes," but the term "reticulocytosis" (though perhaps more accurate etymologically) seems to me less desirable than the shorter and more convenient term.

In this paper I propose to consider the cytological status of these cells, and offer a few experimental and clinical facts pertaining thereto. Though now always studied by some form of so-called vital staining, they were almost surely first observed by Ehrlich<sup>1</sup> in 1881, in air dried blood smears stained with a saturated aqueous solution of methylene blue. To his credit it should be noted that at that time he considered them as not regressive. In using his method with preparations very freshly spread, I have seen the very fine network that he describes, but it is far less satisfactory than the use of vital staining.

Ehrlich's rather cursory observations were not followed up, although Pappenheim was working on the problem in the nineties.† With the beginning of the new century, however, hematological literature produced many observations and discussions on the nature of the various basophilic substances that are to be found in erythrocytes. The consensus of opinion resulting from these studies (though it must be admitted that but little conclusive proof has been brought forward) is that the reticulation indicates a protoplasmic substance (*substantia granulo-filamentosa*) and not a nuclear remnant, (being found well developed in nucleated erythrocytes and staining metachromatically with some nuclear stains) and that it indicates a young blood cell and not an old or degenerated cell. The reasons for the latter belief are that reticulocytes are found in great numbers in young infants and rare in adults, also that they are increased in those diseases in which there is an extra demand and an unimpaired source of supply (as in hemolytic

\*From the Laboratory of Clinical Pathology of the Philadelphia General Hospital.  
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†I have been unable to find this matter discussed in his inaugural thesis (Berlin 1895), to which references have been made. In a footnote on page 54 of an article in *Virchow's Archivk. Pathologische Anatomie*, 1899, clix, he speaks of the use of thionin and neutral red as vital stains, but there is no mention of a reticulum in erythrocytes.

jaundice), and diminished or absent where the demand is lessened (as after transfusions) or if the bone marrow function is damaged (as in aplastic anemia). Stainability of the reticulum in many instances parallels polychromatophilic with the Romanowsky method. Enumeration of the polychromatophilic cells, however, cannot replace the reticulocyte count after vital staining, as the sharp distinction in the latter method does not exist in the former; also the former is not so delicate a method, so that very slight reticulations would be lost, and the count therefore always lower. That the two are not necessarily identical is shown by the fact that many polychromatophilic blasts do not contain reticulum, and that in combined staining, the two are not constantly associated.

The basophilic granules or "stippling," such as found in lead poisoning, are usually considered to be of a different nature and probably indicate an abnormal or degenerated cell. The various nuclear remnants (Howell-Jolly bodies, etc.) belong, of course, to a different category. The very widespread distribution of mitochondria in the protoplasm of animal cells, together with certain similarities in behavior, has led to the assumption that the vital staining reticulum was the mitochondria of the young erythrocyte. Until this has been definitely proved, however, it would seem wiser not to confound the two terms, as is occasionally done; furthermore, Key<sup>2</sup> has recently acquired important evidence tending to show that the two substances are not identical. He calls attention to the fact that the reticulum differs from mitochondria, not only in morphology and various staining reactions, but also in its persistence after shedding, its resistance to heat and to solution by water, acetic and other acids, and alcohol, ether and chloroform.

#### METHODS

As the reticulum can be demonstrated in the erythrocyte by any method that introduces freshly drawn blood cells to any of the vital stains, and displays them suitably under the microscope,<sup>3</sup> it is obvious that many different procedures will be advocated. Having been interested in these cells for a number of years, and tried various methods, we have found the following to be the simplest and most satisfactory for clinical use. A stock 0.3 per cent solution of Brilliant Cresyl Blue (Grubler) in normal salt solution, is prepared in quantity sufficient to last several months, filtered and kept in the ice box to inhibit molds. (Saturated aqueous solutions have also been used). Before using, it is diluted with four (4) parts of a 2 per cent sodium oxalate solution in normal salt solution to one (1) of the stock stain and in this strength is useful for several days. (If Grubler's stain is not available, a 1:2000 aqueous solution of Janus Green is recommended as a stock solution, and similarly diluted before using. I have not yet found an American-made Brilliant Cresyl Blue that is satisfactory.) From a free flowing cut, a drop of blood is sucked into a pipette and quickly diluted about 1:10 with the stain (for this purpose a leucocyte pipette is convenient, taking double the amount of blood customary for a leucocyte count). After standing 10 to 15 minutes, this is well shaken and a wet coverslip prepara-



tion made from the contents, ringed with vaseline (to prevent drying and troublesome convection currents), and examined with the oil immersion lens. (Though for the most part easily visible in lower magnifications, some of the scantier reticula would inevitably be overlooked, unless the oil immersion is used.) This gives about 150 to 200 cells to a field, which can be reduced if desired by inserting a paper with a rectangular slit in the ocular. If reticulocytes are found to be frequent in the preliminary survey, the percentage is obtained by counting the number found in 500—or better 1000—erythrocytes, viewed in at least four different sections of the preparation. If rare—less than 1 per cent—truer results will be obtained if many more cells are examined, 10,000 or more. With good preparations, this can be done with reasonable accuracy by estimating the average number of cells per field, and counting the number of reticulocytes per field, until the requisite number has been examined. Especial care should be taken to prevent crenation, which makes the recognition of the reticulum more difficult and perhaps inhibits the entrance of the dye into the cell. If a permanent preparation is desired, more of the mixture can be spread on a slide, counterstained with a Romanowski stain, and mounted in the usual manner.

The above method seems to have some advantages over that of Vogel,<sup>4</sup> which has been advocated in some text books. It is not only simpler, but ensures a more representative sample. As the reticulocytes are said to have a different specific gravity from the normocytes, any method involving sedimentation would tend to introduce an error on this account.

A still simpler technic for clinical use employs a dry method with coverslips previously prepared with a dried film of the vital stain.<sup>5</sup> I have not found, however, that as high counts are obtained by this as by the wet method. In the haste necessary for making an even spread, it is possible that all the reticulocytes, and especially those with a very slight reticulum, are not properly stained.

The appearance of reticulocytes with Brilliant Cresyl Blue has already been so well described that it can be dismissed here with a reference to the accompanying sketch assembled from a slide prepared in the manner above described (Fig. 1). Attention should be drawn, however, to Key's observation that the form of the reticulum may vary greatly with the stain and method used, so that the familiar forms probably do not picture the structure as it occurs in the unaltered cell, where it probably exists as a diffuse substance. We have also noted that the reticulocytes tend to be larger than the normocytes, and if the slide is allowed to stand, more of them become shadow cells than do the normocytes. This would appear to be contrary to the usual opinion that the younger reticulocytes are more resistant than the adult cells to changes in tonicity of the containing fluid. We have also observed that even in preparations that are not overstained, some of the normocytes assume a greyish green appearance, much darker than the rest. Whether this indicates a significant tinctorial difference in these cells, or whether it is an artefact due to some other factor, such as difference in osmotic pressure, we

are not in a position to decide. Metachromatic granules (staining red by this method) can occasionally be found.

#### EXPERIMENTAL RETICULOSIS IN ARTIFICIAL PLETHORA

An opportunity was offered to study the percentage of reticulocytes in dog's blood during the production of experimental plethora by means of daily transfusions from dog donors over periods of several months.<sup>6</sup> This is similar to but more prolonged than Robertson's<sup>7</sup> experiments with rabbits and has afforded somewhat similar results.

In four dogs studied in this manner, the reticulocytes either vanished entirely or diminished to such a point (considerably less than 0.1 per cent) that the possibility must be considered that those found had been introduced with the slightly anemic donor's blood. The curve of one of these dogs is shown in Table I. The temporary rise in the reticulocyte count immediately

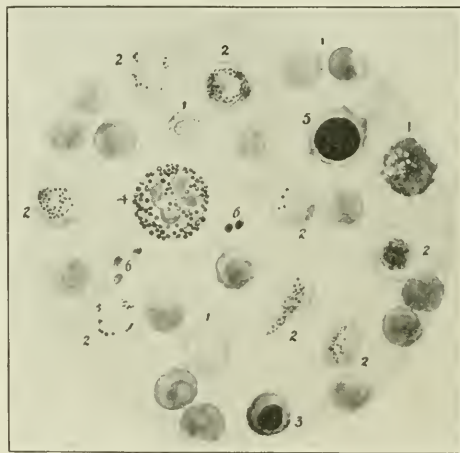


Fig. 1.—Sketch of selected blood cells, vitally stained with Brilliant Cresyl Blue; 1, normocytes one pale, two others crenated; 2, reticulocytes, showing granulofilamentous substance, appearing as a small or heavy network, or wreath; 3, normoblast containing a reticulum; 4, polymorphonuclear leucocyte, with highly refractile granules; 5, lymphocyte with homogeneous nucleus and "glassy" protoplasm; 6, blood platelets.

after transfusions were begun was found in another dog, and considered by us as probably due to bone marrow irritation.

A further record of the value of reticulocytes as an index of blood regeneration is shown in Table II, where dog 20-6, for reasons that we were unable to ascertain, developed a rapid and extreme hemolytic anemia six weeks after daily transfusions of 100 c.c. of whole blood had begun. Though the transfusions were continued without interruption, the hemoglobin count fell from 133 per cent (Newcomer) to 13 per cent in twenty-three (23) days, and the erythrocyte count from 10,000,000 to 650,000 per cu.mm. After ten (10) days, when the hemoglobin had reached 50 per cent, the reticulocytes began to increase and normoblasts and megaloblasts appeared in the peripheral circulation. The number of these fluctuated inversely with the hemoglobin count—the reticulocyte count being the more delicate and the more consistent—until after transfusions had been stopped, the reticulocyte percentage

arose to the extremely high level of 81 per cent. With the gradual recovery from anemia, the reticulocyte count fell again until after four (4) months both curves had reached almost normal levels.

The only instance that I am aware of in which such levels have been surpassed, is in Sappington's<sup>8</sup> rabbits made anemic with phenylhydrazine, where a reticulosis of more than 80 per cent was found in several instances and of 91 per cent in one instance, disappearing in ten days with return of the hemoglobin count to normal.

In dog 20-9 (Table I) a less severe anemia was present from the eighth to eleventh week, and during this period a mild reticulosis developed. It is to be expected that the degree of anemia at which the bone marrow will be sufficiently pushed to put forth an increased number of the relatively immature reticulocytes will vary considerably in different individuals and condi-

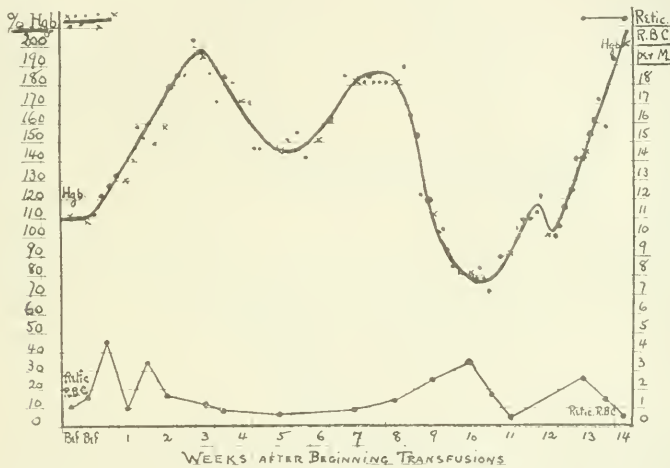


Table I.—Effect of daily repeated transfusions on hemoglobin and reticulocyte count. Dog. 20-9.

tions. With the continuance of transfusions the anemia in this dog was replaced by plethora and again the reticulocytes disappeared from the circulation.

#### NORMAL STANDARDS IN MAN AND LABORATORY ANIMALS

As I have not been able to find any statement of the normal reticulocyte picture in the common laboratory animals, Table III has been prepared to show the averages and normal ranges in normal animals living under customary laboratory conditions. It will be noted that, especially in rabbits, guinea pigs and mice, the individual variation is considerable, and it is quite possible that other individuals of a different strain and living under different conditions, would show still further variations. Lee and Minot<sup>9</sup> have placed the average for man at 0.8 per cent, and in Wood's *Chemical and Microscopic Diagnosis* and Emerson's *Clinical Diagnosis* (5th edition) even higher figures are given. This is distinctly higher than I have found in normal adults, (0.3 per cent) but in view of the individual variations, the matter is sufficiently uncertain for practical purposes, if initial counts are considered abnormal only

if over 1 per cent or less than 0.1 per cent. Qualitatively it may be said that on the whole the reticulum tends to be large and heavy in the species that show the higher percentages. In the monkey it is especially delicate and feathery, while the human reticulocyte stands midway in these particulars, between those of the dog and cat, and those of the smaller animals.

RETICULOSIS IN INFANCY AND DISEASE

The average reticulocyte count for the normal human adult has been discussed in the previous paragraph. Statements as to the normal averages for

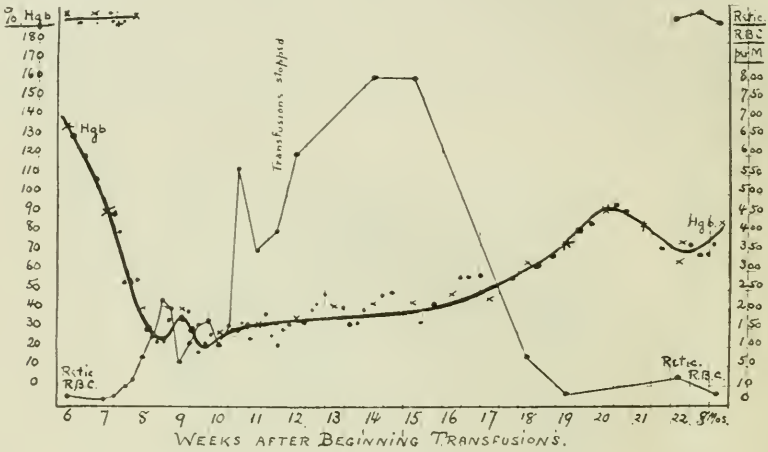


Table II.—Effect of daily repeated transfusions on hemoglobin and reticulocyte count, with supervision of anemia. Dog 20-6.

TABLE III  
PERCENTAGE OF RETICULATED ERYTHROCYTES IN VARIOUS ANIMALS

SPECIES	AVERAGE	NORMAL RANGE
Man	0.3%	0.1—0.8%
Monkey	0.3%	0 —0.8%
Dog	0.6%	0.1—1.4%
Cat	0.2%	0 —0.4%
Guinea pig	3.0%	1.0—4.0%
Rabbit	2.0%	0.6—2.8%
Mouse	4.0%	1 —6.0%

infants, if given at all in text books of clinical pathology, are sufficiently at variance with the facts as I have found them easily to give rise to errors in diagnosis or prognosis. Thus it is usually stated that the average for normal infants is from 5 to 10 per cent, and even 20 per cent has not been considered abnormal. As will be seen in Table IV, I have not found any to exceed 5 per cent. or after the first twenty-four (24) hours to exceed 3 per cent. By the end of the first week, practically normal levels are reached, so that with these exceptions, the normal infant's reticulocyte picture is the same as that of adults. This roughly parallels the hemoglobin and erythrocyte curve for the same period. Cathala and Daunay,<sup>10</sup> the only others who appear to have studied the reticulocytes in early infancy, also found an increased num-



ber at birth, which reached normal levels before the end of the first week. In eight infants they found from 11,000 to 360,000 reticulocytes per cu.mm. at birth; 25,000 to 155,000 the first day; 5,000 to 60,000 the second day; "very rare" by the seventh day; and (with the exception of one icteric) "very rare" by the seventh day. Allowing for the transient erythrocytosis that occurs during the first week of life, this would give a ratio of from 2 to 60 reticulocytes per thousand at birth, 3 to 20 per thousand the first day, 1 to 9 per thousand the second day; "rare" to 3 per thousand the fourth day; and less than one per thousand thereafter; i.e., distinctly lower figures than in our series. Reticulosis, on the other hand, in common with other hematological changes, occurs more readily and to a greater degree in infancy than during adult life.

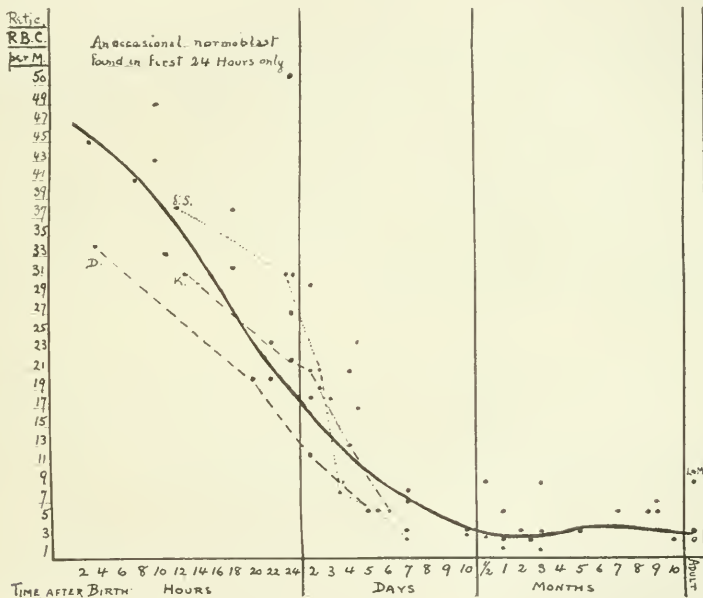


Table IV.—Curve showing number of reticulocytes per thousand during infancy. The solid line indicates the probable average curve; the broken lines denote a few characteristic individual curves.

Variations in the reticulocyte percentage in disease naturally depend on the intensity of the demand and the capacity of the bone marrow to respond. Thus in pernicious anemia, while there is usually a reticulosis of from 2 to 5 per cent during "blood crises" or periods of remission, this may rise to 10 or 15 per cent; while in periods of regression these cells may be completely absent. In the two forms of hemolytic jaundice, the reticulosis is out of all proportion to the severity of the anemia, being customarily as high as 10 and even 20 per cent. The reason for this increase, which is of diagnostic import, has not been determined. In secondary anemias, the reticulosis may be said to be roughly proportioned to the severity of the anemia, and according to Christian,<sup>11</sup> it is also present in the purpuras. In true polycythemia they are also said to be increased, which would support the view that there is an increased demand for erythrocytes in this condition. In aplastic anemia and



other forms of anemia due to decreased blood formation, these cells are diminished (reticulopenia) or absent.

#### SUMMARY

1. Erythrocytes revealing a more or less extensive reticulum (granulofilamentous substance) by the methods of vital staining, may be conveniently designated "reticulocytes."

2. The reticulum is probably of protoplasmic origin, and indicates an intermediate stage between the erythroblast and the adult erythrocyte. A simple method for their recognition and estimation is described.

3. The greater delicacy of the tests for these cells and the greater constancy and delicacy of their variations in the peripheral blood, makes them more valuable criteria of the functional activity of the bone marrow than the study of polychromatophilia or nucleated forms.

4. In the blood of dogs made plethoric by repeated transfusions of blood, the reticulocytes diminished or disappeared entirely during the plethoric stage. With the occurrence of a hemolytic anemia, a reticulosis occurred, which in one instance reached 81 per cent.

5. The average percentage of reticulocytes and their normal range in man and the common laboratory animals is given.

6. The average curve of reticulosis during infancy is given, and the variations occurring in disease discussed.

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# SOME OBSERVATIONS ON BLOOD SUGAR AND THE ALLEGED GLYCOSURIA FOLLOWING OPERATIVE PROCEDURES ON THE THORACIC DUCT\*

BY CARL S. WILLIAMSON, M.D., ROCHESTER, MINN.

RECENTLY in connection with certain studies on the thoracic duct observations were made on the blood sugar values and the alleged appearance of glycosuria following a fistula of the thoracic duct or ligation of the duct in the right pleural cavity.

Lepine and later Biedl maintained that glycosuria develops in a high percentage of instances following fistula of the thoracic duct or ligation of the duct in the pleural cavity. Biedl performed a large number of experiments on dogs. In some he ligated the thoracic duct and in others he made a fistula of the duct. A high percentage of all the animals developed glycosuria within a few hours after operation. The glycosuria continued in some instances until the death of the animal; in others it persisted for a few days and gradually disappeared. Biedl believed that the gradual disappearance of the glycosuria was due to the slow establishment of a collateral circulation of lymph. He was also able to lessen the severity of glycosuria by injecting lymph from a normal dog into the blood stream of a diabetic animal. Tuckett confirmed the work of Lepine and Biedl. Prior to the work of Lepine and Biedl, Gaglio found that if the thoracic duct was ligated or a duct fistula made and the pancreas was removed and glycosuria established, the severity of the symptoms diminished greatly; in fact the urine might even become sugar free. Lymph from a normal dog injected into a pancreatectomized dog also reduces the severity of the glycosuria. Tuckett, in a second publication, retracts his statement that glycosuria results from operative procedures on the thoracic duct and believes that the glycosuria produced by a fistula or ligation of the thoracic duct is such that it could be explained by either the anesthesia or trauma to the cervical nerves during operation.

If the foregoing statements are trustworthy, the importance of the lymph stream as a potential carrier of the internal secretion of the pancreas becomes self-evident. And in view of the fact that the data brought out heretofore are contradictory, it seems advisable to repeat the work.

The data in the present study have been obtained from observations on the dog. All operative procedures were conducted under ether anesthesia, and with sterile technic. The operative technic for fistula of the thoracic duct was that of Biedl with Mann's modifications. Following operation the

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ducts were massaged to keep them open; otherwise they often closed within a week. By careful treatment it was possible to maintain a good flow of lymph from two to three weeks after operation. For ligation of the thoracic duct portions of the sixth and seventh ribs were resected and an opening into the right chest made. The lung was walled back with moist sponges

TABLE I  
BLOOD SUGAR VALUES BEFORE AND AFTER OPERATION

DOG	DATE	TIME	BLOOD SUGAR PER CENT	REMARKS
E 443	3-29-22	8:30 a.m.	0.107	Before operation Thoracic duct ligated After operation; ether hyperglycemia
		9:30 a.m.	0.205	
		4:15 p.m.	0.10	
	3-30-22	8:15 a.m.	0.10	
		4:30 p.m.	0.073	
	3-31-22	8:30 a.m.	0.077	After death
E 788	11-1-21	4:25 p.m.	0.167	Thoracic duct fistula After operation; ether hyperglycemia
	11-2-21	1:30 p.m.	0.096	
	11-5-21	8:15 a.m.	0.10	
	11-7-21	3:00 p.m.	0.103	
E 787	11-1-21	3:15 p.m.	0.117	Thoracic duct fistula After operation; ether hyperglycemia
	11-2-21	1:30 p.m.	0.10	
	11-3-21	10:20 a.m.	0.150	
	11-5-21	8:15 a.m.	0.15	
	11-7-21	3:00 p.m.	0.073	
E 770	10-26-21	2:00 p.m.	0.87	Before operation Thoracic duct fistula After operation; ether hyperglycemia
		2:50 p.m.	0.155	
		8:45 a.m.	0.107	
	10-27-21	5:00 p.m.	0.105	
		8:30 a.m.	0.10	
	10-28-21	8:30 a.m.	0.079	
	10-29-21	8:30 a.m.	0.12	
	10-31-21	8:15 a.m.	0.111	
		4:05 p.m.	0.107	
	11- 1-21	4:30 p.m.	0.144	
		10:45 a.m.	0.10	
	11- 5-21	8:30 a.m.	0.079	
	11- 7-21	3:00 p.m.	0.09	
	11-10-21	9:00 a.m.	0.083	
	11-11-21		0.092	
	11-12-21			
E 568	4- 5-22	3:25 p.m.	0.113	Before operation Thoracic duct ligated After operation; ether hyperglycemia
		4:35 p.m.	0.197	
	4- 6-22	8:30 a.m.	0.137	
		4:30 p.m.	0.122	
	4- 7-22	4:30 p.m.	0.109	
	4- 8-22	9:00 a.m.	0.153	
E 661	4-10-22	10:00 a.m.	0.125	
	4- 5-22	2:45 p.m.	0.092	Before operation Thoracic duct ligated After operation
	4- 6-22	3:10 p.m.	0.082	
		8:30 a.m.	0.119	
	4- 7-22	4:30 p.m.	0.113	
		8:30 a.m.	0.109	
	4- 8-22	4:15 p.m.	0.103	
		8:45 a.m.	0.10	
	4-10-22	1:00 p.m.	0.085	
	4-11-22	8:30 a.m.	0.092	

TABLE I—CONT'D.

## BLOOD SUGAR VALUES BEFORE AND AFTER OPERATION

DOG	DATE	TIME	BLOOD SUGAR PER CENT	REMARKS
E 569	4- 5-22	2:00 p.m.	0.15	Before operation Thoracic duct ligated After operation; ether hyperglycemia
		2:55 p.m.	0.254	
	4- 6-22	8:30 a.m.	0.187	
		4:30 p.m.	0.187	
	4- 7-22	8:30 a.m.	0.103	
		4:15 p.m.	0.136	
	4- 8-22	8:15 a.m.	0.153	
	4-10-22	9:30 a.m.	0.129	
	4-11-22	8:30 a.m.	0.109	
E 142	2-29-22	10:00 a.m.	0.121	Thoracic duct fistula
		4:15 p.m.	0.121	
	3-30-22	8:15 a.m.	0.136	
		4:15 p.m.	0.136	
	3-31-22	9:00 a.m.	0.09	
		4:15 p.m.	0.102	
	4- 1-22	8:30 a.m.	0.11	
	4- 3-22	8:30 a.m.	0.11	
		4:00 p.m.	0.094	
	4- 4-22	8:45 a.m.	0.12	
		4:00 p.m.	0.121	
	4- 5-22	8:30 a.m.	0.121	
	4- 6-22	8:15 a.m.	0.118	
		4:30 p.m.	0.112	

and the duct ligated in two or more places in its course upward from the diaphragm. In making a fistula of the thoracic duct or in ligating it, an anesthesiometer was used to prevent asphyxia should the pleura be opened accidentally in making the duct fistula or intentionally in ligating the duct.

Before operation urine was collected and blood was taken for sugar estimations. A second specimen of blood was taken at the end of the operation, and afterwards specimens of urine were taken once or twice daily. Subsequent specimens of blood for sugar estimation were taken twice daily. Benedict's modification of the Lewis-Benedict method was used for estimating sugar in the blood, and Fehling's method for qualitatively testing for sugar in the urine was employed. Care was taken to collect all data during the time when the ducts were discharging freely. If a duct began to show signs of closing, blood and urine examinations were abandoned.

A fairly wide variation in blood sugar values is shown in the accompanying tabulation. The occasional postoperative increases are not sufficient to be significant. The average postoperative blood sugar value, disregarding the reading obtained immediately after operation is practically the same as the value immediately prior to operation. In some instances it is even a little lower than the preoperative value.

At the time these data were obtained, interesting facts were noted on the relation of the sugar of blood and lymph; these observations will appear later.

Findings with regard to sugar in the urine were uniformly negative before and after operation.

## CONCLUSIONS

1. Practically no changes in the blood sugar level were observed following operative procedures on the thoracic duct, (thoracic duct ligation or thoracic duct fistula).
2. Glycosuria did not develop in any of the animals in our experiments.

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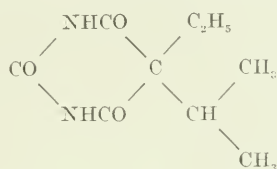
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## THE PHARMACOLOGICAL ACTION OF ISOPROPYLETHYL- BARBITURIC ACID\*

BY D. E. JACKSON, PH.D., M.D., CINCINNATI, OHIO

THE drug used in the experiments performed in the investigation herein described was very kindly given to me by Dr. Lambert Thorp who has made the compound synthetically. Chemically the substance is isopropylethylbarbituric acid, having the following formula:



It is a white, flocculent powder having a melting point of  $197^{\circ}$  C. It is made from ethylisopropyl-malonic-diethyl ester and urea by condensation in the presence of sodium ethylate at  $105^{\circ}$  C. The substance is slightly soluble in cold water, more soluble in hot water and readily soluble in ammonia water, alkaline carbonates and caustic alkalies, forming with the latter the corresponding salts which are readily soluble in alcohol and water, but insoluble in ether. From the aqueous salt solutions, the free acid, melting at  $197^{\circ}$  C. may be reprecipitated by addition of dilute mineral acids. The compound is readily soluble in ether and in alcohol, but insoluble in aqueous solutions of sodium bicarbonate. Microscopically the substance consists of fine white needles which are very stable in the presence of acids. On boiling with sodium hydrate it decomposes into ammonia, sodium carbonate and the sodium salt of ethylisopropyl malonic acid.

Within the limits of medicinal dosage the action of this compound is confined to the central nervous system, and especially to the cerebrum. This action can perhaps best be demonstrated by the administration to dogs by stomach of increasing doses of the acid which has been dissolved in water by the addition of a sufficient quantity of sodium carbonate. Under these circumstances it is found that the drug acts as an efficient and reliable somnifacient. The action of the drug comes on in from twenty to forty minutes, and the symptoms appear to be produced by an overpowering desire on the part of the animal to lie down quietly and go to sleep. At first the animal shows some slight ataxy and unsteadiness in its actions, but when kept well aroused, it appears to readily regain to a considerable extent control over its movements. If let alone, however, it soon lapses into a somnolent state again and falls fast asleep. So far as it is possible to judge from

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the appearance and reactions of the animal this condition bears a very striking resemblance to natural sleep. With moderate dosage the respiration and circulation appear to be practically uninfluenced by the drug. The breathing is regular and of normal depth, and the heart beat is full, regular and apparently of normal force. When let alone, the animal lies quietly and apparently sleeps soundly. But if it be slightly aroused by loud noises or shaking, it slowly opens its eyes, raises its head, gazes blankly about, and then very frequently yawns exactly as a very tired, sleepy child would do, and then, if not disturbed further, it soon lays its head down again and goes

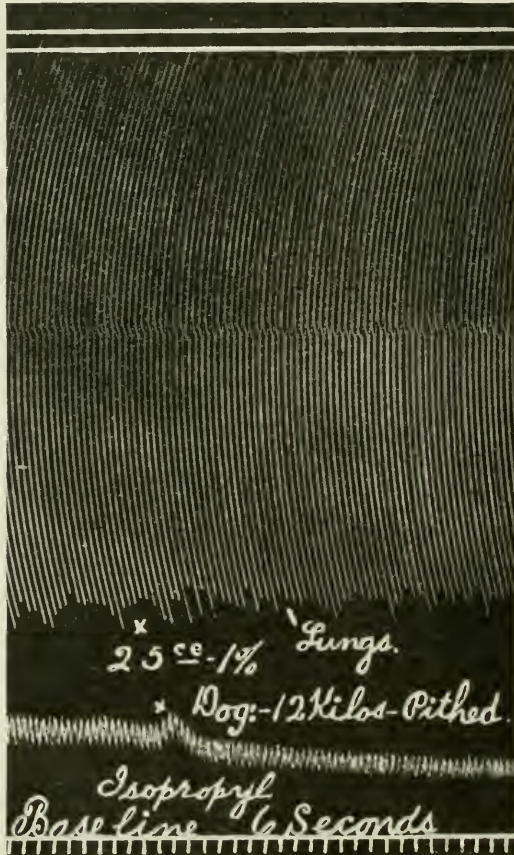


Fig. 1.

back to sleep, as if nothing concerned it except a strong natural desire to sleep and rest. If, however, the animal be more vigorously aroused, even though the sleep had been very profound, it will be found that the animal may appear to wake up and try to get on its feet and move about. The movements are weak, unsteady and incoördinated at first, but with persistent efforts the animal may gain a very considerable degree of control over its movements in a short time and may, in fact, finally be able to get up and move about the room, apparently of its own accord. If not further disturbed, however, it soon lies down quietly again and goes to sleep.

As the dose is increased, the action of the drug tends to appear earlier, and the sleep deepens into a coma from which the animal can be aroused only with great difficulty, or in the deeper stages, not at all. With very large doses, this coma may last for twelve or eighteen hours and the animal may then recover. In some cases animals lived more than forty-eight hours after being given enormous doses, but finally died, perhaps partly from exhaustion and failure to properly take nourishment, as well as from the action of the drug. In the milder cases of prolonged sleep the animals sometimes appear spontaneously to wake up partially, and then they may stretch their limbs, sigh, or very often yawn, and possibly try to move about. Slight muscular tremors frequently are produced by very large doses. These tremors generally first appear in the shoulders or fore limbs, but later may develop in the hind limbs. The tremors appear to be due to a mild stimulation

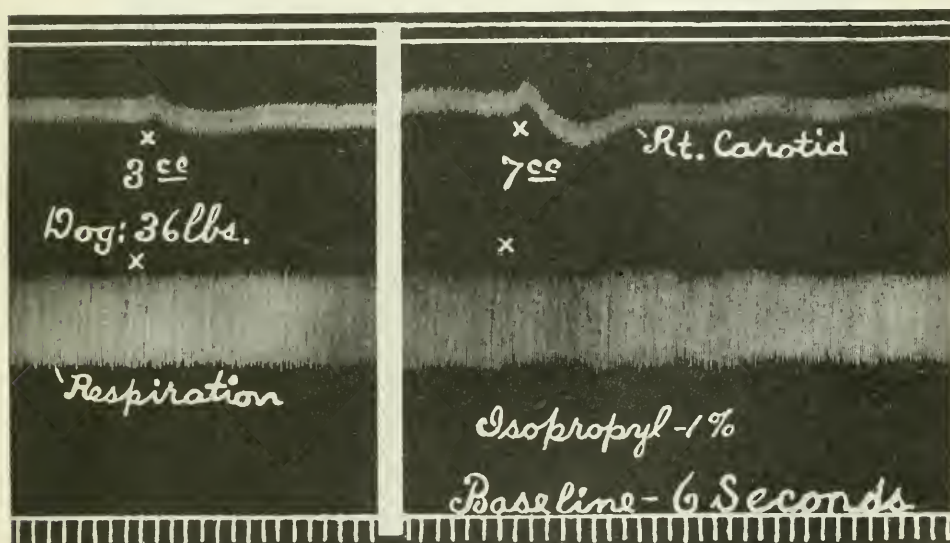


Fig. 2.

of some portions of the spinal cord. They are irregular and intermittent in character, and are usually mild in type. Sometimes twitching may be noticed in the neck or jaw muscles. The tremors may appear at a fairly early stage in the action of the drug in some cases, but they are apparently of but little importance so far as the general action of the drug is concerned. They are of interest, however, because other drugs of this general type have been reported as occasionally causing generalized convulsions. I have watched for this action closely with this drug, but in no case has the slightest tendency toward the development of generalized convulsions been observed. Occasionally in very deep and prolonged sleep from the action of the drug the animal may at times temporarily breathe very rapidly, and occasionally what appeared to be some slight expiratory difficulty has been noted. These actions probably are the result of some slight indefinite stimulation or depression of the medullary, or upper cord, regions by the drug.



There is no bronchoconstriction produced by the drug so far as its direct action on the bronchioles is concerned as is shown in Fig. 1 which represents the blood pressure and lung action in a pithed dog. The excursions in the upper tracing show no change in amplitude when 25 c.c. of one per cent solution of the drug were injected into the femoral vein. (For method of making these lung tracings see Jour. of Pharmacol. and Exper. Therap., 1914, v, 479; also (in detail), Jackson: Experimental Pharmacology, 1917, St. Louis, C. V. Mosby Co., p. 287). Fig. 2 also shows very obviously that ordinary doses have but little or no effect on the respiratory apparatus including the respiratory center in the medulla. In Fig. 2, two separate records are

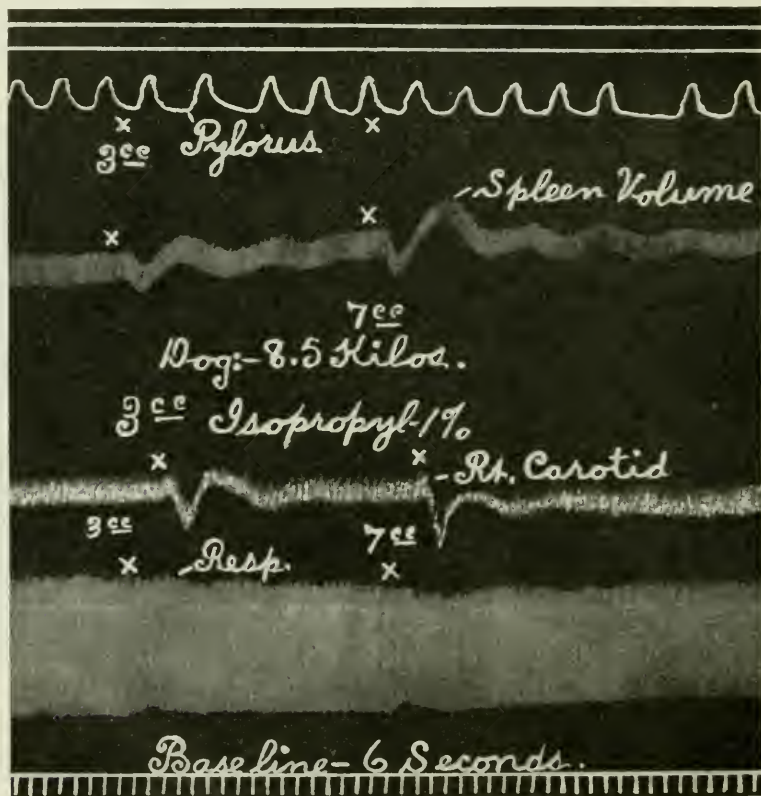


Fig. 3.

joined together to show the effects on the blood pressure and respiration of increasing doses of the drug when injected intravenously. In the first record 3 c.c. of one per cent solution (30 mg. or  $\frac{1}{2}$  grain) of the drug were suddenly injected into the femoral vein. This represents approximately a small therapeutic dose for man. It is seen that the respiration is entirely unaffected as indicated by the respiratory tracing. The blood pressure shows first a very slight rise (from the mechanical increase of the volume of fluid as the solution is suddenly injected into the vein) and then a slight fall. This fall, however, is very insignificant and in two or three minutes the pressure is again back to normal as seen in the second record in which an injection of

7 c.c. is given. This second injection represents 70 milligrams, or proportionately almost twice the therapeutic dose for man. The slightness of the effect on the respiration and the early recovery of the blood pressure indicate that the toxicity of the drug is low. Fig. 3 illustrates the action of the drug on the pyloric sphincter, the spleen volume, the blood pressure and the respiration of a small dog (8.5 kilos). The first injection of 3 c.c. exercises extremely little influence on the regular normal peristaltic contractions of the pylorus. This is significant as throwing some light on the action of the drug on the gastrointestinal system. It would appear that the stomach and intestines are almost entirely uninfluenced by ordinary doses of the compound. The spleen volume is slightly increased by this injection while the respiration is depressed to a very slight degree. The second injection of 7 c.c. (70 milligrams) represents proportionately from two to three times

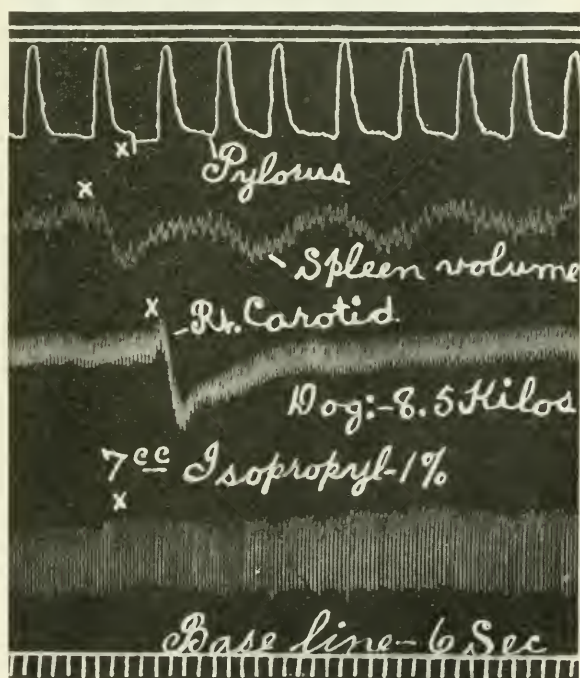


Fig. 4.

the ordinary therapeutic dose by stomach for man. Both of these injections cause slight temporary falls in blood pressure, but this soon again regains almost its normal level. The spleen shows further enlargement by the second injection while the amplitude of the respiratory tracing is somewhat further decreased. There is also detectable a very slight slowing of the respiratory movements near the end of the tracing.

Fig. 4 also shows that the pyloric contractions are not affected by the drug, while the spleen volume tracing takes on an undulating appearance indicating a series of very slight contractions and dilatations. These are perhaps entirely without importance. The respiration shows no immediate



change, but near the end of the tracing some increase in the amplitude is seen. This is of interest as being a very early indication of a phenomenon which becomes much more marked after very large doses; viz., a considerable increase in depth of the respiratory movements as the respiration becomes progressively slower. In this tracing the blood pressure falls sharply following the rapid injection of the drug into the femoral vein, but the pressure soon regains its normal level again, thus indicating that no permanent injury has been done to the circulatory organs.



Fig. 5.

Fig. 5 shows the action of a dose of 15 c.c. (150 milligrams,  $2\frac{1}{2}$  grains) on the spleen volume, blood pressure and respiration. A sharp and rather extensive, but very transient shrinkage in spleen volume occurs. This is perhaps mainly secondary to the sudden, brief drop in blood pressure. But very soon, however, the pressure rises almost to normal again. The depressing effects of the drug on the respiration in this case are more marked and more lasting than those shown in the previous records. This depressing effect on respiration becomes more and more evident with increasing doses and is the final cause of death in fatal cases.

Figs. 6 and 7 should be considered together. They both show the direct action on the heart and on the blood pressure. The animal was etherized, the chest opened and under positive artificial respiration the pericardium was opened and a myocardiograph was attached directly to the heart. The tambour bowl of the myocardiograph was connected by rubber tubing to a recording tambour which wrote on the smoked surface of the kymograph paper. A record of the normal heart tracing and blood pressure was taken and then (Fig. 6) an injection of 3 c.c. of one per cent solution of the drug was injected into the femoral vein. This dosage was approximately proportional to the therapeutic dose in man. It is seen that the heart beat is

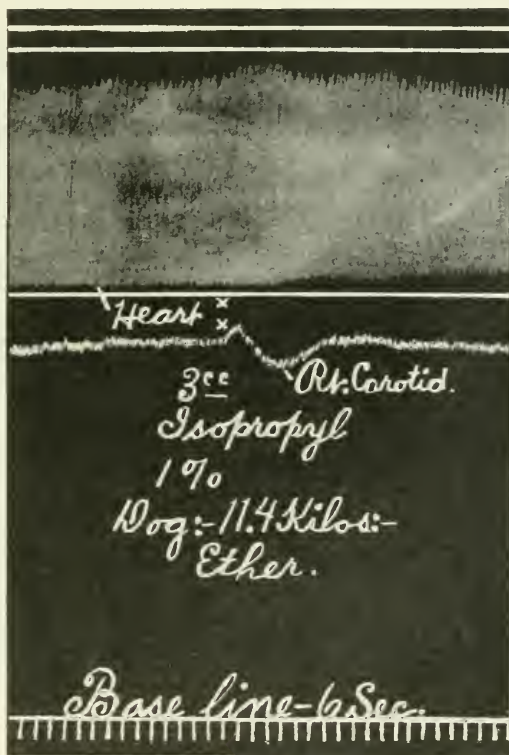


Fig. 6.

but little affected by the drug when the entire dose is injected suddenly directly into the circulation, in which case the compound reaches the heart in its greatest concentration almost at once. In addition the heart in this case was already under the influence of sufficient ether to maintain the animal quietly in a state of anesthesia. This slight preliminary depression from the ether should render the heart more susceptible to the isopropyl compound if it possessed any very marked depressant action on the cardiac muscle. Since the drug under these conditions exercises almost no action in therapeutic quantities on the heart, we may safely conclude that this organ lies almost entirely outside the sphere of action of the drug when given in any

such quantities as would be used medicinally. It may be added in passing that the vagus inhibitory mechanism is entirely unaffected by the drug.

Fig. 7 shows three further injections of enormous quantities into the same animal. Here it is seen that larger doses tend to weaken and decrease the systolic action (down stroke) of the heart while the diastole (up stroke) is slightly increased. But the tendency of the blood pressure to rise again after the fall produced by each injection is very obvious. No such proportionate quantities as these could, of course, be used therapeutically.

I have regularly found experimentally that an animal etherized in the beginning will soon pass under the influence of this compound when a few large injections are given, and then the ether can be entirely removed from

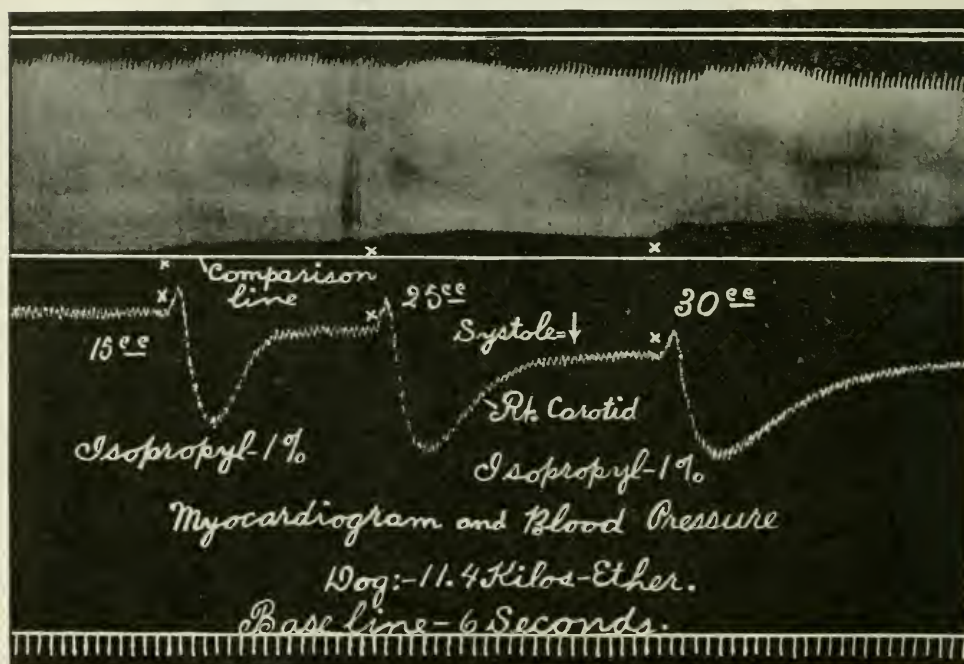


Fig. 7.

the animal. Under these circumstances, the blood pressure and respiration remain in excellent condition. It is often especially advantageous to give one or two fairly large injections of the drug to quiet down an animal which does not take ether well, but continues to breathe rapidly and irregularly and to have slight convulsive seizures, but in which a slight excess of ether tends to immediately stop the respiration or greatly lower the blood pressure. In these cases the reflexes can be quieted down and the animal can be reduced to a calm and satisfactory condition by two or three fair sized injections of the drug. The ether can then be continued without further difficulty.

The final cause of death under this drug is central respiratory paralysis. This is well shown in Fig. 8 in which the respiration stopped spontaneously



after repeated, large injections of the drug. Just before the final respiratory failure the blood pressure had risen to a considerable height as a result of the asphyxial stimulation of the vasomotor centers in the medulla. The kidney volume had also constricted markedly. These two actions show that the circulatory apparatus, both centrally and peripherally, was in very good condition at the time of paralysis of the respiratory center. This is still further shown by the prompt recovery of the heart beat and the blood pressure soon after artificial respiration was started (at the crosses marked "On"). The kidney volume also soon showed evidences of a tendency of the vasculature of this organ to return toward the normal state. The blood pressure does not rise so high after the artificial respiration is started as it had been before the normal respiration ceased, probably because of the asphyxia

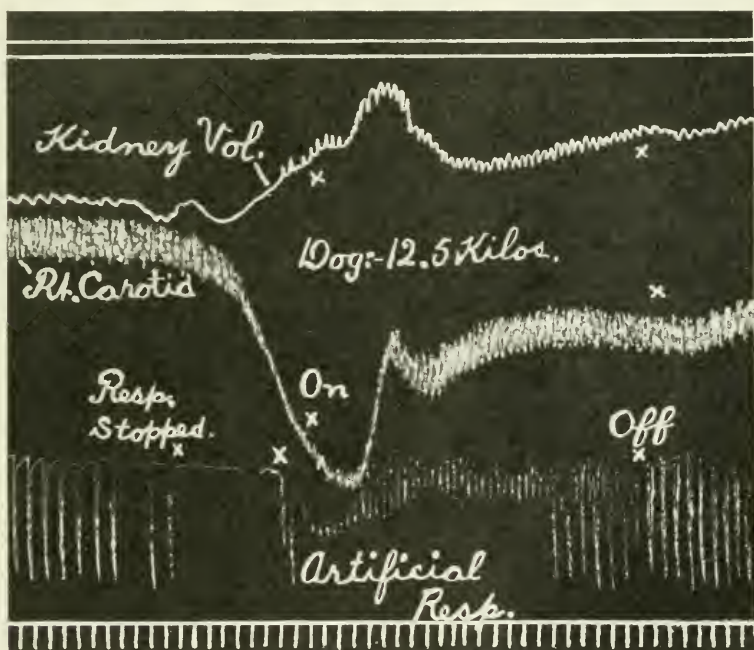


Fig. 8.

which preceded the stoppage of the respiration. After a time it was found that the animal could again breathe spontaneously and then the artificial respiration was stopped. The animal does not recover, however, under these conditions, but after a few minutes again ceases to breathe and dies of central respiratory paralysis. But with care the animal may be repeatedly revived after the respiration ceases, thus showing that artificial respiration is the chief remedy needed in cases of severe poisoning with the drug, but in such cases it is to be noted that the drug is apparently excreted rather slowly.

I have made no special observation regarding the method or form of elimination of the drug from the body, but by the analogy of related compounds, it is very probable that the substance is partly oxidized and partly excreted by the kidneys. I have not noted any special diuresis in dogs fol-

lowing administration of the drug, but it is possible that such action may occur in other animals and perhaps to a slight extent in man. Diuretics, such as caffein, should probably be given in cases of poisoning in man. And respiratory stimulants would also be indicated. From experiments on intact animals it seems obvious that the circulation in general remains in good condition until the respiration has become exceedingly feeble or failed entirely.

I have made no clinical observations under the drug myself, but a considerable number of observations by others (Drs. Lurie, L. A., Cook, R.H., et. al.)\* have shown that the dose (of the acid) which is required to produce a mild sedative effect in the average adult is 2 grains. If deep sleep is desired and the patient is in a highly excited or delirious state,  $3\frac{1}{2}$  grains may be given. These doses may be increased or repeated in case the symptoms call for a more marked depression. Or a large dose (4 or 5 grains) may be given initially (e.g., to maniacal patients) and then small doses (2 grains each) may be given once or twice daily thereafter. These small doses keep the patient calm and enable him to secure quiet, refreshing sleep (Cook, R. H.). The drug (in the form of the sodium salt) may also be administered hypodermically in 2 grain doses two or three times per day. Of the sodium salt (which is very soluble in water) the dose by mouth is two to four grains.

From the therapeutic action of the drug it seems obvious that man is more susceptible to the hypnotic effects of the compound than are dogs. In these animals I have found that the dose beyond which recovery does not occur is in the neighborhood of one grain to the pound of weight of the animal. This would indicate that an average patient of one hundred and fifty pounds weight might survive a dose of nearly ten grams or one hundred and fifty grains. I am inclined to suspect, however, that the fatal dose in man would be smaller than this amount, unless the stomach should be emptied very soon after the drug had been swallowed. The lower degree of susceptibility possessed by dogs, as compared with man, probably holds only in the case of the higher, psychical areas of the cerebrum. And in this case man's higher psychical development probably mainly accounts for his increased susceptibility over that of the dog. So far as the heart and the other circulatory organs and the respiratory apparatus are concerned, it is probable that no great difference exists between man and the dog.

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\*Personal Communications.



# THE EFFECT OF WARM AND COLD WEATHER ON THE BLOOD CATALASE\*

BY W. E. BURGE, AND J. M. LEICHSENRING, CHICAGO, ILL.

IT IS known that oxidation in warm-blooded animals is increased by a fall in the external temperature and decreased by a rise until ordinary room temperature is reached when the stimulating effect of cold on metabolism is reduced approximately to its minimum.<sup>1</sup> This investigation is an attempt to determine the mode of action of cold in increasing oxidation in the body. During the past several years we have carried out experiments in which oxidation in animals was increased as well as decreased in practically every known way. Invariably it was found that whenever oxidation was increased, catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide, was also increased and whenever oxidation was decreased, catalase was decreased. Hence we naturally turned to this enzyme in our attempt to find an explanation for the increase in oxidation brought about by cold weather.

The animals used were rabbits. Catalase determinations were made by adding 1 c.c. of the blood diluted 1 to 3 with 0.9 per cent sodium chloride to 200 c.c. of neutral hydrogen peroxide and the amount of oxygen liberated in 10 minutes was taken as a measure of the catalase content of the blood.

In Table I is shown a comparison of the catalase content of the blood of rabbits in the summer and winter. It may be seen that the blood catalase

TABLE I

The figures after rabbit 1, 2, 3, 4, etc., indicate cubic centimeters of oxygen liberated from neutral hydrogen peroxide in ten minutes by one c.c. of the rabbit's blood diluted 1 to 3 with 0.9 per cent sodium chloride.

RABBIT	BLOOD CATALASE OF ILLINOIS RABBITS	
	SUMMER (74° F.)	WINTER (30° F.)
1	850	1290
2	750	1560
3	700	1080
4	520	940
5	975	1050
6	800	970
7	920	1445
8	940	1220
9	1040	1300
10	830	1175
11	760	1330
12	815	1045
Average	825	1200

\*From the Physiological Laboratory, University of Illinois.  
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in the summer when the average temperature was  $74^{\circ}$  F. was much lower than that of rabbits in the winter when the average temperature was  $30^{\circ}$  F. The average amount of oxygen liberated by 1 c.c. of the diluted blood of the rabbits in the summer was 825 c.c. while it was 1200 c.c. in the winter. These rabbits were kept in the open, winter and summer, and fed on a uniform diet of hay, oats and bread.

Catalase determinations were made of the blood of rabbits for each month from August to April inclusive. These rabbits were also kept in the open, during winter and summer, and fed on a uniform diet. The results obtained

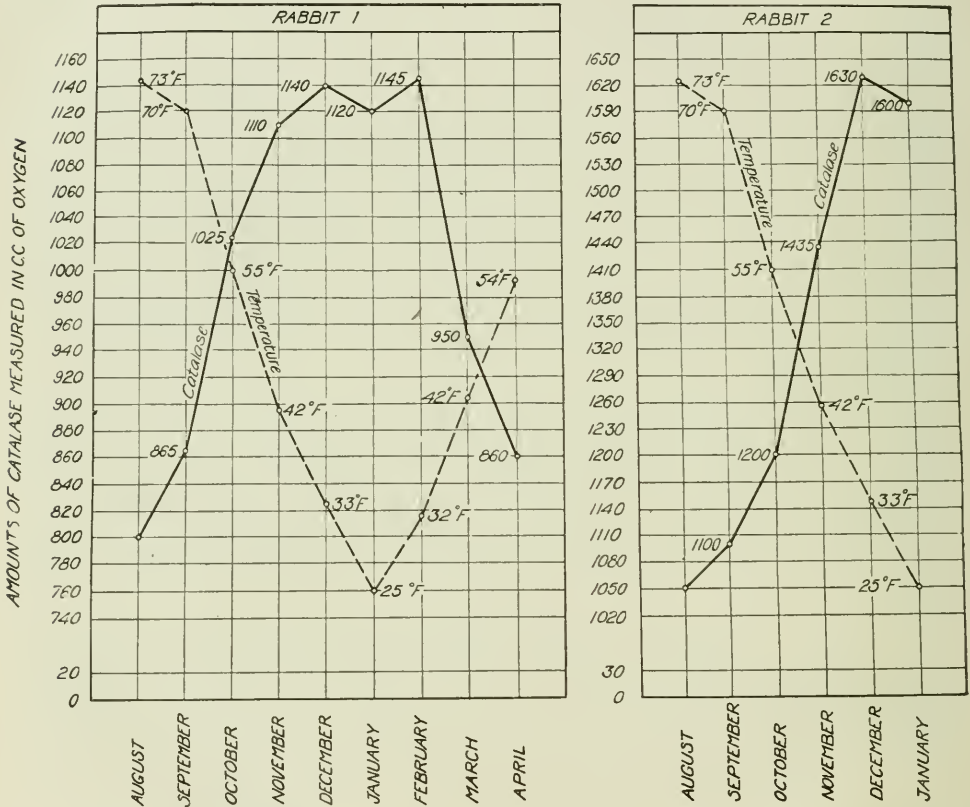


Fig. 1.—Curves showing the effect of a fall in temperature from August to January on the blood catalase of rabbits and of a rise in temperature from January to April.

from two of these rabbits, which are typical, are shown in Fig. 1. It may be seen that the temperature fell from  $73^{\circ}$  F. in August to  $25^{\circ}$  F. in the following January, and that there was an increase in catalase during this period as is indicated by the fact that in August, 1 c.c. of the diluted blood liberated 800 c.c. of oxygen from hydrogen peroxide in 10 minutes, whereas in January, 1 c.c. of the diluted blood liberated 1120 c.c. It may be seen further that when the weather began to grow warmer during the months of February, March and April, there was a corresponding decrease in catalase.

In Table II is shown a comparison of the blood catalase of Illinois and

Louisiana rabbits in December. It may be seen that the temperature in Illinois was 33° F. while it was 70° F. in Louisiana. It may also be seen that the blood catalase of the rabbits of Illinois, where it was cold was considerably higher than those of Louisiana where it was warm. The average amount of oxygen liberated by the blood of the five Illinois rabbits was 1014 c.e. while the blood of the Louisiana rabbits liberated only 759 c.e.

In Fig. 2 is shown the effect on the blood catalase of bringing rabbits from Louisiana to Illinois, in the month of December from Louisiana, where it was warm, to Illinois,

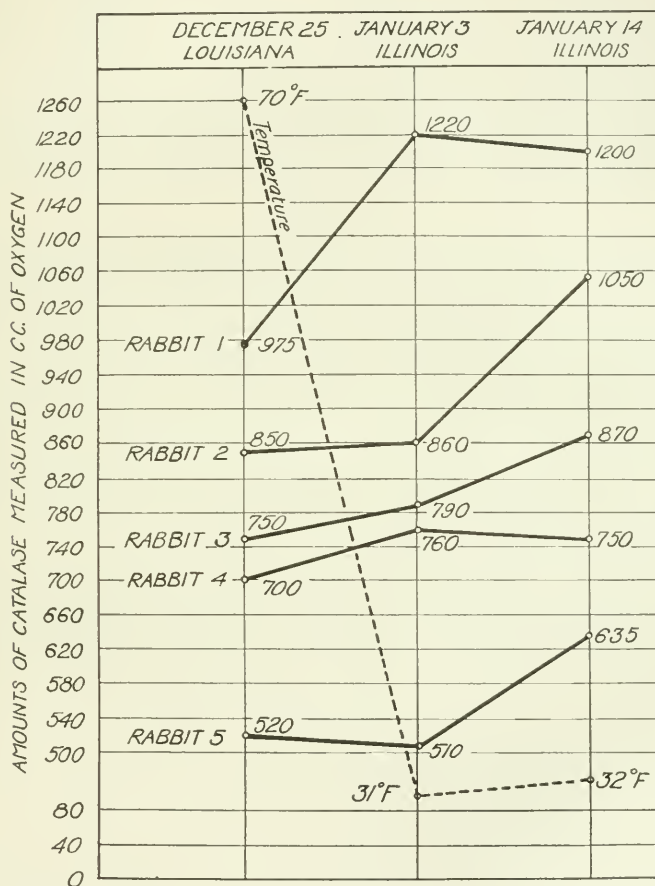


Fig. 2.—Curves showing the effect on the blood catalase of bringing rabbits from Louisiana to Illinois in December.

where it was cold. The average temperature for the month up to December 23 was 70° F. in Louisiana. One c.e. of the diluted blood of No. 1 Louisiana rabbit liberated 975 c.e. of oxygen from hydrogen peroxide in 10 minutes, Rabbit 2, 850 c.e., Rabbit 3, 750 c.e., Rabbit 4, 700 c.e., Rabbit 5, 520 c.e. The rabbits were brought to Illinois and kept there from December 25 to January 3, when catalase determinations were again made. It may be seen that the catalase of the blood of Rabbit 1 had increased a great deal, while Rabbits 2, 3, 4, and 5 had increased very little. From January 3 to January 14, how-

TABLE II

The figures after rabbit 1, 2, 3, 4, 5, indicate cubic centimeters of oxygen liberated from neutral hydrogen peroxide in ten minutes by one c.c. of the rabbit's blood diluted 1 to 3 with 0.9 per cent sodium chloride.

RABBIT	BLOOD CATALASE OF ILLINOIS AND OF LOUISIANA RABBITS	
	ILLINOIS (33° F.)	LOUISIANA (70° F.)
	DECEMBER	DECEMBER
1	1080	750
2	940	700
3	1050	975
4	970	850
5	1030	520
Average	1014	759

ever, the blood of all the rabbits showed a considerable increase in catalase with the exception of Rabbit 4, which showed only a small increase.

## SUMMARY

The blood catalase of Illinois rabbits is lowest in the summer when the weather is hottest and highest in the winter when the weather is coldest.

As the weather grows colder, passing from summer to fall and winter, the blood catalase gradually increases, and passing from winter to spring and summer when the weather is growing warmer, the blood catalase gradually decreases.

The blood catalase of Louisiana rabbits in December is much lower than that of rabbits at the same time in the colder climate of Illinois. However, if the Louisiana rabbits are brought north to Illinois in the winter, the blood catalase rapidly increases.

The increase in oxidation in warm blooded animals brought about by cold weather is attributed to an increase in catalase and the decrease in oxidation brought about by warm weather is attributed to a decrease in catalase.

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# STUDIES ON GASTRIC AND DUODENAL ULCER: THE RELATION OF EPIGASTRIC HERNIA TO GASTRIC ULCER—A CLINICAL AND EXPERIMENTAL STUDY\*

BY JACOB MEYER, M.S., M.D., AND A. C. IVY, M.S., PH.D., CHICAGO, ILL.

EPIGASTRIC hernia is frequently encountered by the surgeon and internist. It is present in about 1 per cent<sup>1, 2</sup> of all patients examined. From Hall's experience with soldiers only about 20 per cent of the cases of epigastric hernia have symptoms and they are those in which omentocoele is present, bowel being present in the hernia only in a very small percentage of the cases.

The symptoms complained of are either obscure abdominal pain or discomfort, a feeling of "dragging" in the epigastrium, or the gastric ulcer syndrome.

The literature on this subject records fifty-four cases of epigastric hernia with gastric symptoms. Of these fifty-four cases, 30 are reported as having had symptoms comparable to those of the gastric ulcer syndrome, but ulcer is proved (blood in feces and vomitus, revealed by operation) only in seven of these cases. (We are liberal when we state that ulcer was proved in seven cases.) In other words, the literature reports that 13 per cent of cases of epigastric hernia with gastric symptoms had a concomitant gastric ulcer.

The early observers Ury,<sup>3</sup> Strauss,<sup>4</sup> and Cohnheim,<sup>5</sup> and more recently Hall,<sup>2</sup> call attention to the possibility of epigastric hernia symptomatology being mistaken for gastric ulcer. But we are of the opinion that with the present methods of history taking and gastroenterological diagnosis a differential diagnosis should not be difficult. On the other hand, we do believe that the marked similarity and relationship between epigastric hernia symptomatology and the gastric ulcer syndrome should be emphasized and kept in mind by the surgeon and internist in the differential diagnosis of gastric ulcer.

Our interest in this condition was aroused by the following case: S. T., age twenty-two, clerk, male, complained of pain in the epigastrium, belching and constipation. Pain was severe, "hunger-like" and gnawing in character, and occurred regularly about two hours after meals, was located in the epigastrium and relieved by alkalies and posture. Physical examination was negative, except for the presence of a small, soft, globular mass about two inches above the umbilicus in the median line. This mass was not reducible and no impulse was obtained on coughing. Gastric analyses showed a free acidity of forty units and a total acidity of one hundred units. A diagnosis of epigastric hernia possibly associated with gastric ulcer was

\*From the Department of Physiology, Loyola University School of Medicine.  
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made and the patient was advised to enter the hospital for further observation. As this was refused, the patient was placed under ulcer treatment as recommended by Sippy. Six weeks later, with no apparent improvement, the patient returned and entered the hospital for examination. At this examination the gastric analyses showed a free acidity of twenty-seven and a total acidity of eighty-one units. Fluoroscopic studies and serial plates of the stomach showed no evidence of any lesion in the stomach.

In view of these observations, an operation was advised to correct the hernia. The operation by Dr. B. F. Lounsbury revealed a small globular mass (one and a half centimeters in diameter) of properitoneal fat that was continuous with the omentum (omentocoele) which protruded through a small defect in the linea alba and was adherent to the parietal peritoneum. Gross inspection of the stomach showed no evidence of ulcer of the stomach. The appendix was removed because of the presence of fecal concretions, but histological examination showed it to be normal. The patient was placed on a full diet for a period of a week after the operation. Two years have elapsed since the operation and the patient is still free from all symptoms.

Because of the apparent comcomitance of gastric ulcer and epigastric hernia with omentocoele, the latter is alleged to be the cause of the gastric ulcer. Kelling<sup>6</sup> offers the hypothesis that the tension exerted by the omentocoele "irritates the sensory nerves of the stomach wall, a secondary stimulus of the sympathetic ganglion results, impulses are originated which produce a narrowing of the arteries; the circulation in the submucosa accounts for the development of the ulcer, or prevents the healing of one when originated by other causes," Strauss<sup>4</sup> reports the opinion that the continual pull exerted by the hernia on the stomach wall causes the development of the ulcer. Ury<sup>3</sup> states that the hernia appeared to be a factor in the development and course of the ulcer in his cases. Soper<sup>7</sup> expresses the opinion that the hernia does not cause the ulcer but prevents the healing of an ulcer produced by some other factor. Hall<sup>2</sup> believes that "long continued reflex irritation of the stomach by the omental 'drag,' so that hyperacidity and ulcer follow, as in chronic appendicitis, produces the ulcer."

Since it is alleged that epigastric hernia with omentocoele is an etiological factor of gastric ulcer and since we have in this condition one that can be easily and accurately duplicated and put to experimental test, we thought it worth while to make an experimental study of epigastric hernia in the dog as it is related to gastric ulcer.

#### EXPERIMENTAL PROCEDURE

An epigastric hernia was produced by the following method in a series of dogs: The abdomen was opened in the mid-line by a two inch incision downward from the tip of the xiphoid process. Gastric omentum about one-half of an inch from the pyloric sphincter and one inch from its attachment to the greater curvature of the stomach was picked up and sutured to the external sheath of the rectus outside the linea alba in such a manner that a "tug" or pull would be exerted on the stomach by every contraction

of the stomach and that free herniation of the adjoining omentum would occur. One inch of the incision was closed by interrupted sutures leaving a defect of about one inch in the abdominal wall. The subcutaneous fat and fascia was then drawn together by interrupted sutures and the edges of the skin approximated by a subcuticular suture. Fourteen dogs were so operated. After operation the animals were examined, x-rayed and autopsied.

#### SUMMARY OF RESULTS

Of the fourteen dogs operated on, thirteen showed a definite and typical epigastric hernia of the gastric omentum (by examination and autopsy) so situated that there would be a "tugging" action exerted on the greater curvature of the pyloric portion of the stomach about one-half to one inch from the pyloric sphincter. In four dogs this condition was present for six months and in others for one to two months. We x-rayed the four dogs which were kept six months to ascertain whether there was a deformity of the stomach caused by the "tugging" action of the herniated omentum and to prove definitely whether or not this tugging actually existed in our experimental condition. Slight deformity of the greater curvature was observed in two of the animals and in all of them a "tugging" action could be demonstrated by pulling on the herniated omentum, thereby causing marked deformity of the greater curvature at the site of attachment. In six animals a definite pocket or pouch formation associated with dense fibrous bands was found at autopsy on the greater curvature of the stomach one inch from the pyloric sphincter at the site of attachment of the herniated portion of gastric omentum. Bone fragments were found in the "gastric pocket" of one of these animals, the stomach being otherwise empty. From these results and observations we are convinced that a definite "tugging" action was exerted on the stomach and some deformity of the stomach was produced by the experimental epigastric hernia.

#### DISCUSSION

Since we have noticed in our experimental animals with epigastric hernia the occurrence of a pocket formation in the wall of the stomach, we might suggest that such a pocket may be considered as a locus minoris resistentiae and a potential site for an ulcer. This would be of greater significance in man than in the dog because of the upright position that man assumes. Also such a factor might lead to the production of an ulcer in man and not in the dog, because gastric ulcer occurs very infrequently in dogs<sup>8</sup> and it is difficult to experimentally produce a chronic ulcer in a healthy dog. On the contrary, such a pocket formation has never been reported to occur in man. Only two cases have been examined fluoroscopically, however, our case and one of Hall's cases.

Since the above suggestion is only speculation, we interpret our negative results as demonstrating that epigastric hernia is not a direct etiologic factor in gastric ulcer. If it were a causative factor, we should at least have got an acute ulcer. If Kelling's or Strauss' hypotheses were true, an

ulcer should have occurred because the mechanisms referred to by them existed in our dogs. Whether such a reflex irritation or vasoconstriction as the various hypotheses infer actually exists is physiologically very questionable.

We prefer to believe that the concomitance of epigastric hernia and gastric ulcer is a coincidence. We explain the percentage (13 per cent) of concomitant occurrence of epigastric hernia and ulcer by the supposition that cases of concomitance of the two conditions are reported because of their interest, whereas cases with epigastric hernia without proven ulcer are not reported because such cases are relatively common.

Our experiments have no bearing on Soper's opinion that the hernia causes a delay in or prevents the healing of an ulcer from some other cause. In our opinion this hypothesis has much in its favor and we expect to put it to an experimental test. Soper's case which failed to respond to treatment until the hernia was operated and repaired, strongly supports his contention.

Our case showed what may be considered by some clinicians as hyperacidity, in the view of the observations of Carlson,<sup>9</sup> Ivy<sup>10</sup> and the contributions of Refuss and Hawk, such findings may be within the physiological variation of normal persons. It is conceivable that an epigastric hernia may cause reflex disturbances which in turn might alter gastric secretion. We are convinced, however, that this does not occur because in our experience with Pavlov pouch dogs in which several epigastric hernias involving the omentum have accidentally occurred, no abnormal acidity or secretion has been observed. It is also to be kept in mind that it has not been demonstrated that hyperacidity is an etiologic factor of gastric ulcer; in fact Dragstedt<sup>11</sup> has shown that hyperacidity is not a causative factor of gastric ulcer.

The character of the pain or distress in our case interested us very much because a gastric ulcer was not present. The pain was almost identical with the pain of gastric ulcer, occurring one to two hours after meals, dull, gnawing and intermittent in character, located in the epigastrium and relieved by soda and food. Cohnheim ascribed the genesis of these pains to the tension exerted on the herniated omentum brought about by the vigorous contractions of the stomach which occur intermittently. We concur in this opinion and believe that it is supported by the fact that alkalies will relieve both types of pain, the pain of gastric ulcer and of epigastric hernia with omentocele, the alkalies relieving the pain according to Carlson<sup>12</sup> by causing an inhibition of the vigorous tonic contractions, which begin one to two hours after meals and continue until the stomach is empty then to disappear until a hunger period ensues. This opinion is further supported by the fact that coarse food excites and enhances the pain in cases of epigastric hernia (Soper<sup>7</sup>).

#### SUMMARY

We are unable to demonstrate experimentally in dogs that epigastric hernia with omentocele is a causative factor of gastric ulcer. We tacitly suggest that the tendency to a local pocket formation in the wall of the

stomach brought about by the "tugging" of the omentocoele may be an etiologic factor of gastric ulcer in man. We prefer to believe that concomitant gastric ulcer and epigastric hernia in man is coincident and not that the hernia has a direct etiologic relationship to the ulcer. Epigastric hernia does not cause hyperacidity, as the gastric findings in such cases are within the normal variation. We are convinced, as are all others who report cases of epigastric hernia with gastric symptoms, that operation is definitely indicated.

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# THE RELATION OF FIBROSIS AND HYALINIZATION TO LONGEVITY IN CANCER\*

(A STUDY OF 194 CASES)

WILLIAM C. MACCARTY, M.D.,† ROCHESTER, MINNESOTA

IN June, 1921, I published a series of observations on the relation of local lymphocytic infiltration and cellular differentiation to the length of postoperative life in gastric carcinoma.<sup>1</sup> While making those observations no special attention was paid to the relation of fibrosis and hyalinization to postoperative life. It was not done because both fibrosis and hyalinization

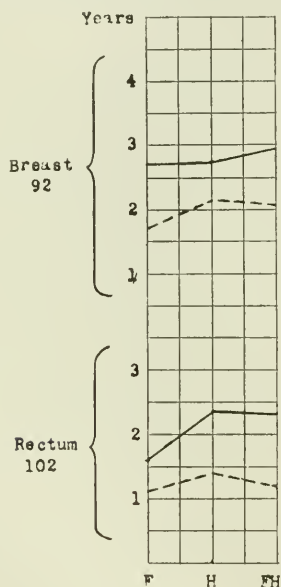


Fig. 1.

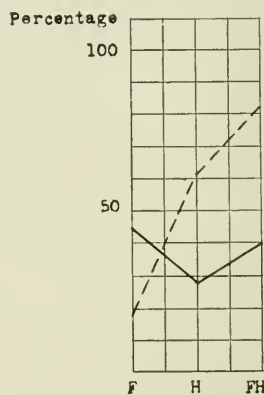


Fig. 2.

Fig. 1.—Average length of postoperative life with and without factors.

Solid line, duration of life of cases with the factor. Dotted line, duration of life of cases without the factor.

Fig. 2. Percentage increase of postoperative life with factors checked against postoperative life without factors.

Dotted line, rectum. Solid line, breast.

are rather infrequent in the series of gastric carcinomas which has come under my observation. There are, however, two organs in which these factors (Figs. 1 and 2) are more frequent and it was thought that a study of these might throw some light on the problem of the defensive mechanism in cancer. Two series of patients, all of whom had died of recurrence or metastasis following radical resection, were studied:

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The following generalizations may be made:

1. The frequency of fibrosis in association with cancer of the breast and rectum is practically the same in both organs.

2. The frequency of hyalinization in association with cancer of the breast is greater than in cancer of the rectum.

TABLE I  
OBSERVATIONS IN 194 CASES

	BREAST	RECTUM
Cases	92	102
Average length of postoperative life	2.5 years	1.47 years
Frequency of associated fibrosis	68 per cent	75 per cent
Frequency of associated hyalinization	52 per cent	2 per cent
Frequency of associated fibrosis and hyalinization	52 per cent	2 per cent
Average length of postoperative life with associated fibrosis	2.72 years	1.53 years
Average length of postoperative life without associated fibrosis	1.87 years	1.29 years
Average length of postoperative life with associated hyalinization	2.81 years	2.33 years
Average length of postoperative life without associated hyalinization	2.21 years	1.44 years
Average length of postoperative life with associated fibrosis and hyalinization	2.89 years	2.33 years
Average length of postoperative life without associated fibrosis and hyalinization	2.05 years	1.28 years

3. When fibrosis is present in association with cancer of the breast and rectum the postoperative length of life is increased 34 per cent.

4. When hyalinization is present in association with cancer of the breast and rectum the postoperative length of life is increased 40 per cent.

5. When the two factors (fibrosis and hyalinization) are present in combination in association with cancer of the breast and rectum the postoperative length of life is increased over 56 per cent.

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# LABORATORY METHODS

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## A SIMPLE TECHNIC FOR THE PREPARATION OF A SPINAL (HEADLESS) ANIMAL\*

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BY REGINALD ALEX CUTTING, M.A., PH.D., CHICAGO, ILL.

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VARIOUS technics have been devised for the preparation of spinal animals for purposes of research in physiology and pharmacology, as well as demonstration before classes in these subjects; they fall into two general classes, (1) those which depend upon the use of surgical procedures, such as pitling and gross decapitation, and (2) those which depend upon the use of drugs, as, for instance, the injection of chloroform into the vertebral artery (Jackson) or carotid artery (Luckhardt) or directly into the fourth ventricle (McGuigan and Heinekamp). The latter methods make a special appeal to pharmacologists not only because the procedures involved are relatively simple and the results generally satisfactory, but more important still, the attainment of the end by pharmacological means is intrinsically a mental satisfaction. The physiologist, on the other hand, and especially the physiological pedagogue, hesitates to use drugs in procedures in which the same end can be attained without them since their use unnecessarily obscures an already complicated picture. The use of chloroform, or any other drug, as an agent for the destruction of the higher centers of the central nervous system is, accordingly, from the physiologist's point of view open to this general criticism, which in case of experiments where beginners in physiology are concerned, may amount to the difference between pedagogical success and failure, and, furthermore, to the specific criticism that the action of the drug introduced in any of the ways already described cannot be properly controlled; even with careful technic the injection of chloroform into the cervical arteries may not be without cardiac effects, and if the drug be injected directly into the fourth ventricle it becomes impossible to say with certainty the exact level to which the effect may be extending, indeed, in the latter case, it becomes somewhat a matter of chance whether so important a center as that governing respiratory movements is or is not destroyed by the mere mechanical passage of the needle. Not only so, but the matter of technical simplicity which the pharmacological methods favor is apt to be more apparent than real since one must take into consideration the fact that the operation of tracheotomy, as a minimum, must be performed for purposes of artificial respiration whatever the method used.

As between pitling and gross decapitation the former method has the dis-

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\*From the Physiological Laboratory of the College of Medicine of the University of Illinois, Chicago.

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advantage that, while almost as radical as the latter surgically, the site of the operation is, in this case, more or less obscure both during and after the operation and, further, the procedure may be inhumane since it leaves the part of the animal central to the point of section essentially uninjured unless the blood supply to the head be cut off or the brain itself destroyed, procedures in the first case amounting technically to decapitation and in the second case involving uncontrollable hemorrhage if thoroughly performed.

The process of injecting starch granules into the cephalic circulation as a mechanical means of destroying the brain substance is ingenious, but has little besides its ingenuity to recommend it.

Taking into consideration these factors, and many others which might be mentioned if space permitted, the original operation of decapitation (Sherrington) seems to be by far the best technic for routine work on the spinal animal and should be the method of choice for most special purposes as well. The decapitate preparation is not difficult to make; the following method has been used by the author for three or four years and has proved simple and serviceable; it entails so little manipulation that, in the case of the common laboratory animals, cats, dogs, rabbits, etc., the entire operation including the maintenance of anesthesia can be performed by a single individual in less than half an hour.

The animal should be preferably a male and in good physical condition. Ether anesthesia is induced in the usual manner and is maintained with the ether bottle after tracheotomy; the incision for the latter should extend from the lower margin of the cricoid cartilage nearly to the suprasternal notch. Starting from the laryngeal end of this incision two secondary skin incisions are carried upward and backward to encircle the neck and meet dorsally in the median line at the level of the atlanto-occipital articulation; in making these incisions one should avoid cutting so deeply as to injure the superficially placed external jugular veins.

The cervical margin of the skin, including the deep fascia, is now dissected up and turned back for several centimeters, thus exposing the musculature of the neck. The closed blunt points of a pair of long curved hemostatic forceps are next plunged under the anterior musculature at the level of the third cervical vertebra, entering them just behind the external jugular vein on the left, working them through just anterior to the vertebral column, and bringing them out on the right side also just posterior to the external jugular vein; the end of a strong waxed cord is now caught between the jaws of the forceps, and the latter is withdrawn, thus pulling the cord through under the musculature. The cord is tied tightly about the muscle mass without including the trachea; this manoeuvre shuts off the blood supply through the two carotid arteries and the external and internal jugular veins.

The hemostats are inserted at the same point as before, but this time they are worked through the musculature posterior to the vertebral column and are brought out in the median line at the level of the third cervical vertebra; a ligature is passed and tied in the manner previously described. The corresponding muscle group on the right side is also tied off. In tying these ligatures care should be taken to draw the cord very tight, for their function is to



cut off blood supply; a convenient form of knot is the common "slip-knot" secured with a "half-hitch."

At this point 5 c.c. of a 1-10,000 solution of adrenalin in normal salt solution is injected subcutaneously in the flank region, the ether supply is cut off, and artificial respiration introduced, and at the same time heat is supplied to the body by means of an electric heating pad, a bank of incandescent electric lights, hot water bottles, or the like.

The vertebrae are now to be occluded; to do this the hemostats are inserted successively beneath each of the muscle groups already tied, drawing the cord through in such a way as to encircle the spinal column; the ligature is tied tightly, working it down firmly between the second and third cervical vertebrae.

The apex beat will be feeble after this procedure, but should be readily palpable; in case it is not the last ligature is to be loosened and 3 c.c. more of the adrenalin solution injected; in this case the ligature is again tightened at the end of five minutes.

At the end of five minutes, if the heart still continues to beat properly, the head is severed from the body above the ligatures, the incision being carried through the vertebral column between the atlas and occiput; this can be done quickly with the aid of an amputating knife.

After wiping away the blood which drains back from the head and stopping any small hemorrhages which may occur from the cervical stump, the latter is covered with gauze or cotton and the skin flaps are brought together and secured with hemostats.

The blood-pressure rises slowly; reflexes may be expected to return gradually at the end of twenty minutes.

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## ARTIFACTS IN BLOOD CULTURE PLATES SIMULATING COLONIES\*

BY WILLIAM THALHIMER, M.D., MILWAUKEE, WIS.

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IN a blood culture taken recently with a new Roux syringe, colony-like structures developed which simulated exactly colonies of *Streptococcus viridans*. It required a great deal of labor to determine that the structures which appeared to be colonies were artifacts. This is the second time that I have had this experience and I have seen this same phenomenon twice in blood cultures taken at Mt. Sinai Pathological Laboratory, New York City, which Dr. E. P. Bernstein kindly showed me. The cases from which my own blood cultures were taken were febrile cases where there was a possibility of positive blood cultures being present. These apparently positive blood cultures consequently caused considerable anxiety until they were proved to be negative. I, therefore, considered it sufficiently important to determine the nature of these struc-

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\*From the Laboratory of Pathology, Columbia Hospital, Milwaukee, Wisconsin.  
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tures which simulated bacterial colonies, and, perhaps, others who have been similarly misled may profit by the results of my experience.

My first experience was with a blood culture taken at the University of Virginia Laboratory of Pathology in 1910. It was only after my second experience that I recalled that the previous apparently positive blood culture had also been taken with a Roux syringe. In both instances, the syringe was new and it occurred to me that perhaps some minute particles in the new syringe became mixed with the blood and gave rise to the development around them of colony-like structures. The Roux syringe has a rubber plunger and a glass barrel encased in metal. Upon the new rubber there is a thin layer of yellowish or cream colored powder, the exact constitution of which I do not know, but which, I believe, contains sulphur. It occurred to me that the fine particles of this powder might cause the phenomenon referred to. The pseudocolonies, in each instance, developed slowly. A few appeared in twenty-four hours, a moderate number in forty-eight hours and all were present by seventy-two hours. By transmitted light on the blood agar plates, the colonies appeared as minute, slightly greenish specks. Immediately around these specks there was an area of clearing from one-half to one millimeter in width and immediately around this a second area twice as wide as the first one which was definitely greenish in tint and which shaded gradually into the surrounding medium. The pseudocolonies were target-like in appearance. By reflected light, these structures were dull gray in color. Under the low-power of the microscope, the speck at the center of these pseudocolonies was translucent in appearance and had a bluish color. The speck was irregular in outline and did not have the structure of a bacterial colony. Many colonies were fished from the plates in each blood culture and numerous smears from these were examined and many subcultures were made on various types of media (bouillon, agar, glucose serum agar, blood agar, etc.). In no instance was growth obtained in the subcultures nor were bacteria demonstrated in the smears. In both blood cultures the bouillon flasks remained sterile.

The deductions made above as to the cause of the colony-like artifacts were proved to be true by the following simple experiment:

A new rubber plunger from a Roux syringe was boiled in normal saline solution and some of the powder of the surface was scraped off with a sterile platinum loop and mixed with a tube of human blood agar at 45°. This was poured into petri dishes and controls were also poured of the blood agar containing none of the scrapings. The plates were then compared and the control plate remained sterile and showed no structures at all resembling colonies. In the plate of blood agar mixed with scrapings from the rubber plunger, colony-like structures identical with those described above appeared in small numbers in twenty-four hours. In forty-eight hours, hundreds of these structures appeared. Many of these structures were fished, subcultures made and smears were also examined. No bacteria could be demonstrated by either of these methods.

Many blood cultures have since been made with the original syringe after

the powder of the surface had been thoroughly cleaned off and no colony-like artifacts have appeared in any of the plates.

It is well recognized that minute specks may gain entrance into a blood culture, and into the blood plates which are poured, and that sometimes these specks will cause structures very similar in appearance to colonies to develop around them. These specks undoubtedly vary in constitution, so that various types of specks may give rise to this phenomenon. In the two instances recorded here the powder from the surface of the rubber plunger of the syringe was undoubtedly responsible for the pseudocolonies. In view of this, it is hoped that the findings recorded above will not prove uninteresting.

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### PRESERVATION OF COMPLEMENT SERUM WITH 25 PER CENT SODIUM CHLORIDE\*

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By M. W. LYON, JR., M.D., AND VERA B. TRAGER, SOUTH BEND, INDIANA.

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THE preservation of complement serum with sodium chloride has been advocated by many workers. Kolmer, Matsunami and Trist† in an elaborate piece of work have reviewed the subject of complement preservation and performed numerous experiments to determine suitable methods for its preservation. They showed that the most successful method of preserving complement serum is by the addition of 17 per cent sodium chloride and keeping the serum cold.

It occurred to us that the addition of more sodium chloride might be more effective and that the greater dilution required to make the solution of complement and salt isotonic would not require the addition of so much, if any, isotonic salt solution in complement-fixation tests to make a satisfactory bulk for the reagents used. Apparently the largest amount of sodium chloride that serum will hold in solution is 25 per cent. One cubic centimeter of complement serum containing this amount of salt diluted to 30 c.c. yields a 1:30 dilution of complement in an isotonic salt solution. The sodium chloride strength is 0.833 per cent instead of the more usual 0.850 per cent. If diluted 1:29.4 the resulting percentage of salt is 0.85 per cent.

Complement serum preserved in this manner proved so satisfactory that it has been used by us for over a year in complement-fixation work.

The bottle of salted serum, along with control serums, is wrapped in a towel and placed in contact with the ice of the refrigerator. A thermometer inserted in the folded towel registers between 4° and 5° C. By neglect of ice-man on one occasion the folded towel containing a bottle of some fresh complement serum was left on the floor of the ice box closet and remained at room temperature for 24 hours. Its hemolytic activity was apparently unaffected as shown by subsequent titrations. Being curious to see how well salted comple-

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†Am. Jour. Syph., October, 1919, iii, 513-540.



ment serum would keep at room temperature, the next lot of complement serum, collected by heart puncture from eight guinea pigs and salted to 25 per cent, was divided into two parts. A small portion was kept at room temperature and titrated on the regular Wassermann test days along with the bulk of the serum kept on the ice. The table shows the hemolytic unit of the diluted complement serums on successive days, using 0.1 c.c. of a 10 per cent suspension of chicken cells. The chicken blood is obtained from a poultry butcher twice a week and obviously never comes from the same chicken. The cells of individual chickens apparently vary in their resistance to specific amboceptor and complement so that it not infrequently happens that a succeeding titration shows a smaller complement unit than a preceding one. Not every lot of complement serum keeps its activity as well or as long as the one shown in the table.

After the eighteenth day the complement kept at room temperature appeared so weak that no further titrations were made with it. On this day a two millimeter loop of the salted serum kept on ice and another of that kept at room temperature were planted on agar slants. In the case of the room temperature serum, about 100 colonies of a Gram-positive nonpigment forming staphylococcus developed in the incubator. In the case of the ice box preserved serum no colonies developed after 24 hours' incubation, but after several days at room temperature seven colonies of a Gram-negative deep yellow pigment producing bacillus developed. On the twenty-eighth day similar cultures were made. The room kept serum showed essentially the same result as on the eighteenth day. In the case of the ice box kept serum only two colonies developed after several days' maintenance at room temperature, one the same yellow producing bacillus and the other a large nonsporogenous Gram-positive bacillus producing no pigment. The presence of bacteria in preserved complement serum is probably a factor in its deterioration.

No special tests to determine the loss of specific fixation or the acquirement of nonspecific fixation properties were made by us, but an experience of a year's use of complement serum saturated with sodium chloride have yielded results in the Wassermann tests done with three antigens in agreement with clinical findings, with histories, and in a number of instances with the results of Wassermann tests made by others. In laboratories having comparatively few Wassermann tests to do it is probably better to use a preserved complement serum obtained from several animals rather than fresh complement obtained from a single animal.

#### SUMMARY

Complement serum preserved with 25 per cent sodium chloride, that is to point of saturation, and kept on ice retains its activity for hemolysis with specific amboceptor with comparatively little change for from three to four weeks and is satisfactory in performing the Wassermann test. The same salted serum kept at room temperature retains such activity for about 10 days. Bacteria can exist in serum saturated with sodium chloride even though such serum is kept on ice. It is not improbable that the presence of bacteria in preserved complement serum is a factor in its deterioration.



Table showing hemolytic activity of complement serum preserved with 25 per cent sodium chloride, some kept on ice and some kept at room temperature, diluted 1:30.

Days since collecting and adding 25 per cent sodium chloride and smallest quantities of diluted serum needed to hemolyze 0.1 c.c. of 10 per cent sensitized chicken erythrocytes.

	4	7	11	14	18	21	24	28	days
Salted serum kept on ice	0.2	0.15	0.15	0.2	0.15	0.2	0.2	0.3	c.c.
Salted serum kept at room temperature	0.2	0.15	0.25	0.35	0.5				

## A GASOMETRIC METHOD FOR THE DETERMINATION OF UREA NITROGEN IN THE BLOOD\*

(Preliminary Communication)

BY A. MIRKIN, PH.D., NEW YORK CITY

UREA can give off under suitable conditions three different gases: ammonia, nitrogen and carbon dioxide. An accurate method based upon the determination of either one of them would be superior to any colorimetric or titrimetric method from the standpoint of the clinician as such a method would greatly simplify and shorten the procedure. This accounts for the popularity which the so-called hypobromite method is still enjoying, although the inaccuracy and unreliability of the latter have been sufficiently demonstrated. Even still less accurate gasometric methods, as the decomposition with Millon's reagent or with nitrous acid, were used to some extent for the "quantitative" determination of urea.

There is a tendency to attribute the persistency with which many clinicians refused, and some still refuse, to heed the chemical objections raised by chemists to such methods to a lack of chemical training among medical men. This is not quite true. In his work, the clinician must have at his disposal methods capable of yielding quick and reliable results and the procedure must be as simple as possible. Between two methods of which the one is cumbersome and slow, and the other simple and quick, the clinician is liable to choose the latter even if its accuracy is questioned from many sides.

In many cases of nephritis, the urea nitrogen is so far above the normal that the inaccuracy of the method could not disguise it. And for a long time, the determination of urea nitrogen was considered only in nephritic cases. But not all the nephritic cases have abnormally high urea nitrogen; and we know, at the present, that a moderately high urea nitrogen is met with in many other diseases which cannot be characterized as those of renal involvement.

The present technic of urea nitrogen determination based upon the decomposition of urea with the aid of urease and colorimetric or titrimetric

\*From the Department of Laboratories, Beth Israel Hospital, New York City. Dr. Max Kahn, Director.

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determination of the ammonia formed leaves no excuse to the clinician for using the notoriously inaccurate hypobromite method, even if the latter is braced up, remodeled and introduced in its new shape as capable of yielding results within the limits of permissible error.<sup>1</sup> We do not wish to question the accuracy of Andresen's figures, but even with the most perfected apparatus for the determination of evolved nitrogen, the fact still remains that the reaction between urea and hypobromite is neither quantitative nor specific; i.e., other nitrogenous compounds present in the blood gave off nitrogen under the same conditions as urea.

While the urease-aeration-(or distillation) Nesslerisation technic deprives the hypobromite method of *raison d'être*, it suggests itself a gasometric method which has one of the advantages of the former—specificity of urea—and at the same time is simpler and quicker. Instead of determining the  $\text{NH}_3$  of the  $(\text{NH}_4)_2\text{CO}_3$  formed through the action of urease, the carbon dioxide can be determined as well by using the well-known Van Slyke's apparatus for the determination of the carbon dioxide combining power or a more suitable modification. As the blood contains a certain amount of carbon dioxide, this would have to be determined first and deducted from the amount found by decomposing the bicarbonate of the blood incubated with urease.

Partos<sup>2</sup> describes an apparatus for the determination of the carbon dioxide content of the  $(\text{NH}_4)_2\text{CO}_3$  formed from urea through incubation with urease. With this apparatus he determined the urea in the urine and checked up the results with the titration method. The smallest amount of urea which he determined in the urine was 7.56 mg. He also determined the urine in a pure aqueous solution, the smallest amount used in the determination being 1.65 mg. The amount of urea nitrogen in 1 c.c. of normal blood is almost 0.1 of the last named figure, but we saw no reason why even such a small amount could not yield correct results and we proceeded to satisfy ourselves to that effect after a careful scrutiny of available literature failed to disclose that this had already been tried by somebody else.

The following contains a description of the technic we used and of the results obtained so far.

#### Technic

A. Test for total  $\text{CO}_2$   
 2 c.c. plasma  
 1 c.c. urease

B. Test for preformed  $\text{CO}_2$   
 2 c.c. plasma  
 1 c.c.  $\text{H}_2\text{O}$  (free from  $\text{CO}_2$ )

Incubate A and B for about ten minutes at 45-50° C. Cool with running water to room temperature. Run exactly 1.5 c.c. of A into Van Slyke's apparatus, add 0.5 c.c. of distilled water free from  $\text{CO}_2$  and determine the carbon dioxide in the usual way. Do the same with B. For calculation, use the following formula:

$$\text{Ur}_{100} = 63.6 V_1 - V_2 \text{ Wpt.}$$

$\text{Ur}_{100}$  = the amount of urea nitrogen per 100 c.c. of blood.

$V_1$  = Volume of  $\text{CO}_2$  yielded by A.

$V_2$  = Volume of  $\text{CO}_2$  yielded by B.

Wpt. = Weight of 1 c.c. of  $\text{CO}_2$  at the temperature and pressure prevailing during the test.

$$63.6 = \frac{\text{NH}_2\text{CO.NH}_2}{\text{CO}_2} \times \frac{\text{N}_2}{\text{NH}_2\text{CO.NH}_2} \times 100 = \frac{2800}{44} = 63.6$$

<sup>1</sup>Andresen, Gad: Biochem. Ztschr., 1919, xcix, 1.

<sup>2</sup>Partos: Biochem. Ztschr., 1921, ciii, 292.

In Van Nostrand's Chemical Annual (1919, page 100) will be found a table giving the respective weights of  $\text{CO}_2$  at different temperatures and pressures.

It is needless to say that care in carrying out the procedure is of the utmost importance. The apparatus must be absolutely airtight, the stop-cocks well greased, and all the precautions recommended when carrying out gasometric determinations must be painstakingly observed. The reading must be taken as soon as the level of the mercury in the calibrated part of the apparatus becomes steady. One must raise the hand holding the large bulb with mercury so that it should, as nearly as possible, be on the same level with the mercury in the calibrated part of the apparatus. Not more than 0.01 c.c. of caprylic alcohol should be added to prevent foaming; an excess makes the exact reading more difficult.

COMPARISON OF RESULTS OBTAINED BY BOTH THE GASOMETRIC AND COLORIMETRIC METHODS.  
MGMS. OF UREA NITROGEN IN 100 C.C. OF BLOOD.

Colorimetric Method	Gasometric Method
19.1	19.5
18.4	18.9
25.7	26.3
30.1	29.6
49.0	49.6
55.0	56.0

I wish to thank Dr. M. Kahn, director of the chemical laboratory, Beth Israel Hospital, for his courteous and ever-ready help extended to me during the work carried out.

## A SUBSTITUTE FOR THE LONG ROLL KYMOGRAPH\*

BY FELIX P. CHILLINGWORTH, M.D., BOSTON, MASS.

THE necessity of producing several long roll kymographs for student use and the high cost of the same resulted in the writer's evading the issue by having the following type of large drum made.

This drum fits accurately the Harvard kymograph motor, and having a circumference of 90 cm. and a height of 30 cm. results in a greatly increased area of smoked writing surface. The ends of the drum are made of light wood, through which the sleeve passes. The height of the drum permits the use of a wide kymograph paper which has distinct advantages, namely—by an adjustment upward or downward of the drum, the writing point level can be readily changed, which does away with the prevalent method of either raising or lowering the kymograph in its entirety, or on the other hand, of raising or lowering the recording apparatus.

\*From Physiological Laboratory, Tufts Medical School.  
Received for publication, Feb. 11, 1922.



Fig. 1.—Shows drum in position upon the Harvard Motor.

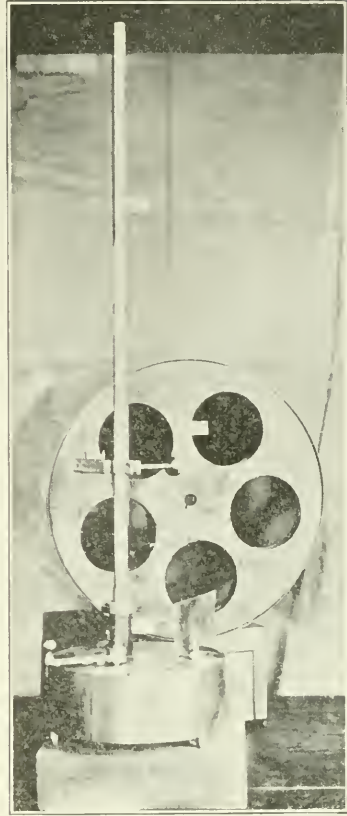


Fig. 2.—Shows the sleeve with the rack and pinion adjustment. 1. Indicates the sleeve. 2. Construction of end of drum. 3. Pinion. 4. Brass bushing.

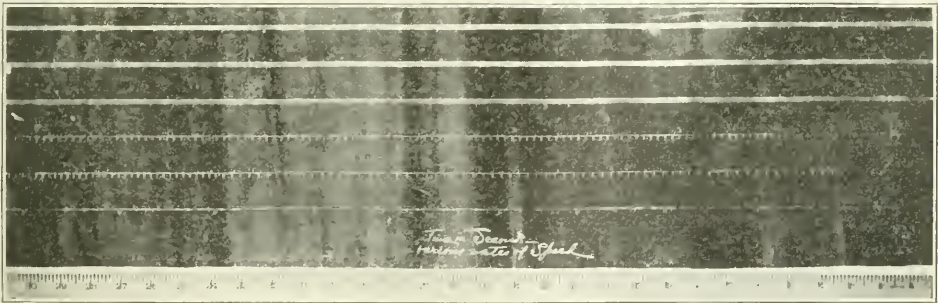


Fig. 3.—Shows photograph of a tracing made to demonstrate the accuracy of this drum. The time being in seconds, records made at various rates of speed.

This adjustment is extremely simple and is brought about by a rack and pinion, the rack being soldered on the sleeve of the drum. This allows instant adjustment in either direction of the drum. The lower end of the sleeve is covered with a brass bushing of sufficient height so that the base of the drum will just clear the governing fan. For some speeds it is desirable to increase the fan surface.



The total cost of this drum was as follows:

1/32" sheet aluminum (90 x 30C) .....	\$1.80
rack .....	.72
pinion .....	.15
<b>TOTAL</b>	<b>\$2.67</b>

Several of these drums were made and have been in use in this laboratory for over a year and have proved most satisfactory.

## THE PRACTICAL VALUE OF AN ICE-WATER BATH FOR USE IN THE COMPLEMENT-FIXATION TEST FOR SYPHILIS\*

BY H. VIRGINIA LANGWORTHY AND E. JANE KERLEY, ALBANY, N. Y.

THIS paper is merely a preliminary report of some work done at the State Laboratory to compare the degree of complement fixation which takes place at different temperatures: that is, room temperature, ice box temperature and ice-water bath temperature. The object of the work was to show the practical value of an ice-water bath for use in laboratories not equipped with a brine-cooled cold-room.

The range of temperature of an ordinary ice box is between 11° and 16° C. and may approach 18° C. if the ice box is opened too frequently. The brine-cooled refrigerator in use in the State Laboratory maintains a temperature of approximately 3° – 6° C. An ice-water bath, however, can be kept at a temperature as low as 2° C.

Brine-cooled ice box temperature (3° – 6° C.), ordinary ice box temperature (12° C.), ice-water bath temperature (2° C.) and room temperature (20° C.) were used to test a limited number of serums received for the complement-fixation test for syphilis. The majority of these were from cases under treatment for syphilis at Auburn Prison, sent to us by Dr. Heacox to whom we are greatly indebted for his hearty cooperation. The others were either from cases showing clinical evidence of syphilis, or from inmates of prisons or reformatories. All of the specimens tested had given marked or slight fixation in the routine test.

Seventy-one specimens were tested with the plain alcoholic antigen, fifty-eight specimens with the cholesterinized antigen. The effect of the different temperatures of fixation was more evident in the tests with plain alcoholic than with cholesterinized antigen. In the tests with plain alcoholic antigen, but one-third of the specimens tested gave the same fixation at all four temperatures. In the test with the more sensitive, cholesterinized antigen, two-thirds of the specimens tested gave the same fixation at all four temperatures.

\*From the Division of Laboratories and Research, New York State Department of Health, Albany. Augustus Wadsworth, M.D., Director.

Presented at the One Hundred and Sixteenth Annual Meeting of the Medical Society of the State of New York at Albany, April 19, 1922.

Received for publication, June 14, 1922.

TABLE I

COMPARISON OF FIXATION IN THE ICE-WATER BATH AT 2° C. WITH FIXATION IN EACH OF THE FOLLOWING: BRINE-COOLED ICE BOX AT 3° — 6° C., ORDINARY ICE BOX AT 12° C. AND ROOM TEMPERATURE 20° C.

NUMBER OF SPECIMENS TESTED	WITH PLAIN ALCOHOLIC ANTIGEN 71		WITH CHOLESTERINIZED ANTIGEN 58	
	Number	Per cent	Number	Per cent
Equal fixation at all temperatures	23	32.39	45	77.58
Unequal fixation at different temperatures	48	67.60	13	22.41
Greater fixation at 2° than at 3° — 6° C.	6	8.45	3	5.17
Greater fixation at 2° than at 12° C.	29	40.84	6	10.34
Greater fixation at 2° than at 20° C.	47	66.19	12	20.68
*Less fixation at 2° and 3° — 6° than at 12° or 20° C.	1	1.40	1	1.72

\*Differences in these two cases very slight, between negative and plus-minus, and between plus-minus and one plus.

Comparing the fixation in the ice-water bath and brine-cooled ice box, the difference is slight. Six, or less than 10 per cent of the entire number tested with plain alcoholic antigen, and three, or only 5 per cent, of the number tested with cholesterinized antigen gave greater fixation at 2° than at 3° — 6° C. The difference in fixation between the ice-water bath and the ordinary ice box is more marked. Twenty-nine, or 40 per cent, of the number tested with plain alcoholic antigen and six, or 10 per cent, of the number tested with cholesterinized antigen reacted more strongly at 2° than at 12° C. The difference in fixation between ice-water bath and room temperature was much more pronounced. Forty-seven, or 66 per cent, of the specimens tested with plain alcoholic antigen and twelve, or 20 per cent, of the specimens tested with cholesterinized antigen gave greater fixation at 2° than at 20° C.

The degree of variation between the reactions obtained at 2° and 3° — 6° was very slight. That between 2° and 12° was slightly more marked, while that between 2° and 20° was in some cases striking, with such differences as + at 2° and  $\pm$  at 20°, or + at 2° and negative at 20° C.

The results given were obtained in an improvised ice-water bath, consisting of a small insulated (17 × 11 inches) copper water-bath encased in a wooden packing box lined with heavy paper. The three-inch space between the outside of the bath and the box was filled with flake asbestos. The bath rested on one-inch excelsior pads. By using blocks of ice that nearly filled the lower well of the bath, a temperature of 2° C. and lower was kept for more than four hours. (Nineteen tests with each antigen, or thirty-eight tests altogether could be placed in this bath.)

A household ice box could be easily converted into an ice-water bath. A wooden one lined with zinc having inside measurements 12 by 17 inches and 12 inches deep is sold for thirteen dollars. The outlet pipe of an ice box of this type could be stoppered, the space under the shelf nearly filled with a large piece of ice, and sufficient water added to cover the wire shelf to a depth of 1½ inches. Such a water bath should keep a temperature of 2° or below for four hours or longer, and should prove of great practical value where a brine-cooled cold room is not available.

## AN AUTOMATIC DISTRIBUTING APPARATUS\*

BY LEON H. CORNWALL, M.D., AND GEORGE PHILIPP SCHMITT, NEW YORK CITY

THE apparatus illustrated in the accompanying photographs is designed to facilitate the performance of work in laboratories where a large number of serologic, immunologic or chemical tests are routinely performed. Its function is to distribute rapidly a measured amount of any required liquid ingredient to a large number of test tubes or other containers.

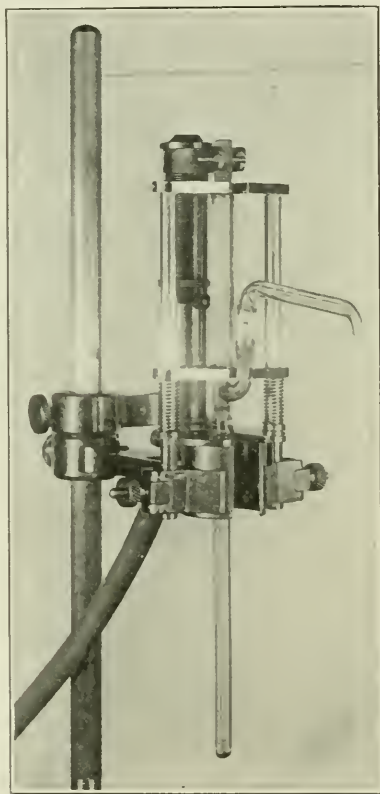


Fig. 1.—Enlarged view of the distributing apparatus illustrating the detailed points of construction.

The apparatus consists of two separate sections, one of glass through which the measured liquid is aspirated and delivered, and the other of metal for the automatic operation of the first.

The glass section consists of tubing of the approximate size and calibre of the ordinary 1 c.c. pipette. There is a vertical portion and a curved horizontal portion. In both the vertical and the horizontal portions there are

\*From the Pathologic Laboratories, City Hospital, New York City.  
Received for publication, June 5, 1922.

bulbous dilatations, each of which contains a conical glass bead. The lower ends of these bulbous dilatations are conical and ground so that the beads fit tightly into them. By this arrangement the beads function as opposing valves. When one valve is open the other is closed and vice versa. This allows fluid to be aspirated into a syringe that is connected to the upper end of the vertical arm and delivered through the curved horizontal arm. The syringe may be fitted into a ground aperture adapted therefor and removed when desired or it may be a continuation of the vertical tubing.

The operating mechanism is of metal and on the principle of a vertically disposed syringe. The piston, instead of being operated by traction, is elevated by air forced into the barrel from a bulb that is manipulated by the operator. The piston descends by gravity when the pressure on the

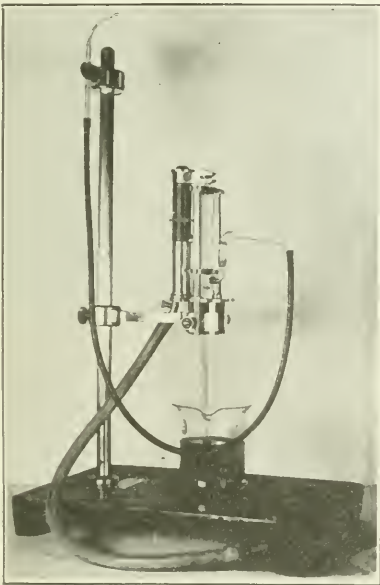


Fig. 2.

Fig. 2.—Showing the distributing apparatus attached to a ring stand and arranged for the distribution of antigen for the Wassermann reaction.



Fig. 3.

Fig. 3.—Showing the distributing apparatus attached to a bottle of colloidal gold reagent.

bulb is released. On one side of the metal piston there is a lug which projects through a longitudinal slit in the barrel. The outside of the barrel is threaded and has a nut which may be screwed up or down. The excursion of the piston is regulated by the adjustment of the movable nut against which the lug on the piston impinges when air is forced in. An arm projects from the upper end of the metal piston and engages the upper end of the glass piston.

The whole apparatus may be attached to a ring stand or clamped to the neck of a bottle or flask. The metal frame to which the glass portion is clamped is light and easily movable. When it is not expedient to utilize the operating mechanism an ordinary syringe or an automatic syringe (see



Jour. Am. Med. Assn., Feb. 18, 1922, p. 506) may be attached to the capillary valve tubing.

When maintenance of sterility is essential the glass parts may be sterilized by boiling and the vertical tube can be inserted through the stopper of a bottle or flask. Contamination may be avoided by flaming the glass tip before use and then sealing it by some simple device such as hot paraffin.

This apparatus will distribute all of the ingredients in serologic work in less than one half of the time ordinarily consumed. It is especially useful for the addition of colloidal gold solution to a large number of tubes. All of the parts are easily detached and assembled. When it is necessary to use clean glassware for each ingredient and simple washing with saline does not accomplish sufficient cleansing the glass section can be removed and a new one inserted in a few seconds. The several parts can be obtained separately and with one operating section any desired number of glass sections may be used.

This apparatus may be obtained from the Will Corporation, Rochester, New York. Trade name "VOL-U-METER."

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## A CONTINUOUS WATER STILL FOR A BACTERIOLOGICAL LABORATORY

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BY MERLIN L. COOPER, CHICAGO, ILL.\*

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THIS continuous all glass water still was devised for use in a bacteriological research laboratory. The advantages of the still are that it utilizes but one source of water for both condensing and distilling; maintains a constant water level in the flask of boiling water by automatically feeding the flask with hot water as needed to replace the water evaporated; runs continuously with little attention; and has a capacity of 1 liter of distilled water per hour for each flask connected into the system.

As shown in Fig. 1 the operation of the still is as follows: Fresh water from the water line passes through glass tube *A*, enters condenser *B*, passes out through glass tube *C* and enters beaker *X* through one of its side arms *D*. From this beaker a small amount, as is required, passes out through another side arm *E*, then through bent connecting tube *F*, and enters the second beaker *Y* through one of its side arms *G*. From this beaker (*Y*) the water, as it is needed in the flask, passes out through side arm *H*, bent glass tube *I*, and enters flask *Z* through side arm *K*.

The water from the condenser, which is not needed to maintain a constant water level in beaker *Y* and flask *Z*, passes out of the first beaker (*X*) through its third side arm *N* to the waste drain through glass tube *O*.

Beaker *X* should be just a little higher than beaker *Y*, and beaker *Y* a

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\*Research Bacteriologist, Morris & Co.

Received for publication, March 20, 1922.

little higher than flask *Z*. Side arms *D* and *G*, supplying the water to their respective beakers, should each be slightly higher than the other side arms.

The water in beaker *Y* is maintained at a steaming temperature by conduction of heat from the boiling water in flask *Z*. In this way this water, as it enters the flask to replace the water evaporated, is already at a high temperature and the boiling of the water in the flask is not interrupted.

In this laboratory a second flask, the same as flask *Z*, was quite often attached to beaker *Y* through side arm *P* and a bent glass tube similar to *I*. This required the use of another gas burner and condenser as the only pieces



Fig. 1.—A continuous glass water still.

of extra apparatus. The water supply for this flask was derived from the hot water in beaker *Y*, the same as for flask *Z*. This double flask system gave a capacity of 2 liters of distilled water per hour. More flasks could be attached to beaker *Y* by attaching more side arms to this beaker, thus increasing the capacity. The same supply of cold water could be utilized for several condensers by having as many of them attached one with another as is found efficient in maintaining the condensers sufficiently cold to condense all steam.

## A CLASS DEMONSTRATION OF INTESTINAL ACTIVITY\*

BY A. E. GUENTHER AND HOMER C. LAWSON, OMAHA, NEBR.

OF ALL the ordinary class demonstrations none presents such uncertainty in results as the one dealing with intestinal movements. The usual "immersion method" first used by van Braam Houckgeest<sup>1</sup> in 1872, in which the abdomen of an animal is opened under a suitable saline solution seldom yields more than an insignificant activity. As a demonstration method to students the immersion method has the great disadvantage that comparatively few observers can gather about the tank to view the movements, if present. More than a year ago, the authors, under the necessity of demonstrating the effects of various drugs upon intestinal activity, conceived the plan of preparing a light adjustable board, provided with a central opening through which the intestines of an operated rabbit might hang. The board serves as a support to the body of the animal and can be lowered until the pendent intestines enter a saline solution in a jar placed beneath the board. The intestines buoyed up by the solution can be viewed by a class of ordinary size from any point within the confines of an ordinary class room. The demonstration is improved if a flat anatomical preparation jar of clear glass is selected and the intestines are illuminated by the use of a properly shaded incandescent light.

The method gave disappointment at times when the intestines failed to hang down into the solution owing to the buoyancy imparted to them by contained gas. It served, however, as the point of departure of a series of trials which eventuated, finally, in the method to be described and which gives so striking a demonstration of intestinal activity that all having witnessed it are impressed. The method to be described requires the cooperation of two persons in the preparation for a demonstration.

### METHOD

The animal is anesthetized preferably with a permanent anesthetic. Urethane is satisfactory for rabbits. After removing the abdominal hair an incision is made in the median line approximately four and one-half inches in length, beginning one inch posterior to the tip of the xiphoid and involving only the skin. The skin is then separated from the underlying abdominal muscle by separating the fascial planes, avoiding hemorrhage. The dissection should extend about one inch anteriorly and posteriorly beyond each end of the incision and laterally from one and one-half to two inches in both directions. An eight inch ligature is now drawn through the adjacent right and left edges of the skin incision about one and one-half inches from its

\*From the Pharmacological Laboratory of the University of Nebraska College of Medicine, Omaha. Received for publication, July 5, 1922.

anterior end and allowed to lie in place. (Fig. 1, B, lig.) An incision in the linea alba, opening into the abdominal cavity, should be made somewhat anterior to the center of the skin incision and not exceeding three-quarters of an inch in length. With caution a pair of forceps may now be introduced through the slit towards the animal's left and a loop of the small intestine drawn upwardly and out through the opening. With the fingers, previously moistened, and, exercising care, ten to twelve inches of the intestine may be gently drawn through the opening and allowed to rest on the exterior of the abdominal wall. It is possible, as a precaution, if obstruction of the intestinal lumen is feared, to introduce into the slit and fasten in place a hard rubber ring two centimeters in diameter, preliminary to drawing out the intestines. This, however, was not found essential to the success of the demonstration.

If the intestines are found to be empty and flat, it is desirable, at this

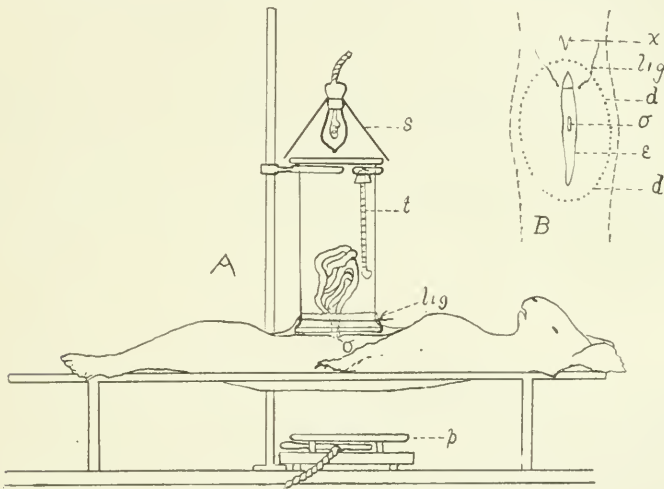


Fig. 1.—(A) Sectional diagram of apparatus to demonstrate intestinal activity. *t*, thermometer; *s*, shaded light; *o*, opening into abdomen; *p*, electric warming plate.

(B) Diagram of operative field. *x*, xiphoid cartilage; *lig.*, ligature; *o*, opening into abdomen; *i*, incision in skin; *d*, area of separation of skin from underlying musculature; *e*, edge of incision into skin.

time, to introduce at various points along the loops a small quantity of air by means of a hypodermic needle. This will render the loops of the intestine buoyant, when later, they are immersed in saline solution. A cylindrical museum jar, three and one-half by seven inches and provided with flanges at base and top, is almost filled with Tyrode's solution which has previously been brought to the temperature of the animal's body. A short thermometer, inserted into a cork to serve as a float, is placed in an inverted position in the jar.

The assistant now grasps the forelegs and ears of the rabbit with one hand and the hind legs with the other and places the animal over the jar so that the intestines hang into the solution. While the assistant thus holds the animal in place, the operator draws the loosened skin over the flange of the jar and stretches it in place by means of the ligature mentioned above.



(Fig. 1, A, B, lig.) It will now be necessary to remove the layer of air separating the abdominal wall from the solution in the jar. To do this the operator inserts a rubber tube between the anterior edge of the incision and the jar and by means of a funnel at the other end of the tube introduces warmed Tyrode's solution which displaces the air. By suitable manipulation all of the air may be expelled easily.

By proper cooperation between the experimenter and the assistant the jar and the animal are rapidly inverted. This maneuver is made possible by the experimenter placing one hand on the back of the animal while the other hand grasps the jar and holds it in constant contact with the body of the animal while the inversion is being made. The inverted jar is now held by a suitable ring support so that its weight and that of the contained column of liquid do not bear down on the body of the animal. The buoyant intestinal loops float up into the jar and can be viewed by a large circle of students. The effect is greatly enhanced by slightly darkening the room and illuminating the intestines from above by means of an incandescent bulb as shown in the illustration. (Fig. 1, A.)

It should be borne in mind that the intestines are not under hydrostatic pressure but under normal atmospheric pressure. Neither need the junction between the skin and the jar be water tight. The relations between jar and rabbit are precisely those existing between an inverted tumbler of water and the sheet of paper which, by atmospheric pressure, serves to keep the water from falling out.

#### EXPERIMENTAL RESULTS

After placing the intestines in a jar, a variable period of time ensues during which the intestines are practically inactive. The period of inactivity, in rabbits, varies from one-half to two hours. In the interval between the second to the fifth hours the activity reaches a maximum but periods of marked activity alternate with periods of relative quiescence. Rhythmical segmentation with alternate flushing and blanching of the segments may be observed in some instances. Peristaltic waves, more or less pronounced, are common. The most frequent and striking movement, however, is the swaying of the loops from side to side, usually complicated by peristalsis. The swaying or pendular movement is, undoubtedly, more pronounced than in the closed abdominal cavity owing to the free manner in which the loops are poised in the solution. Upon these intrinsic movements of the intestine are superimposed the rhythmical thrusts of the pulse and respiration. Occasionally borborygmi are clearly audible at a distance of three to five feet from the preparation. A surprising and unusual spectacle is the drawing of the intestinal wall over the stationary fluid contents within the lumen. That the contents are stationary is established by the presence within the fluid of suspended darker masses plainly visible through the illuminated translucent intestinal walls. Taken all together, the movements are puzzling and complicated but in the course of an hour it is usually possible to witness the typical activities described by Starling and Bayliss.<sup>2</sup>

Although the transportation of intestinal contents (with the exception of the to and fro movements) is not clearly appreciable, it obviously must take place since there is an accumulation of material at the distal end of that portion of the intestines lying outside of the abdominal cavity. Peyer's patches clearly visible in the intestinal wall do not take an active part in the contraction of the walls of the gut, and, consequently, stand out from the general contour of the wall as well defined nodules. The flattening of the intestines when empty is worthy of mention.<sup>3</sup> In one experiment on a rabbit carotid blood-pressure tracings were made during the course of a demonstration. The results are shown in Table I.

TABLE I  
SHOWING EFFECT OF DEMONSTRATION ON BLOOD PRESSURE IN A RABBIT

TIME	BLOOD PRESSURE	REMARKS
2:00 p. m.	72 mm. Hg.	Four hours after urethane administration
2:30 p. m.	60 mm. Hg.	Two. mins. after operation and intestines in jar
3:30 p. m.	66 mm. Hg.	Intestines quite active
8:30 p. m.	66 mm. Hg.	Intestines slightly active

During ordinary summer days the temperature of the solution in the jar as indicated by the enclosed thermometer remains practically constant. The maintenance of the temperature is mainly due to conduction from the body of the animal. On cold days this source of heat may be reenforced by the heat from the incandescent lamp above and by the cautious use of an electric hot plate under the animal board.

This method of demonstrating intestinal movements is of particular service in pharmacology. During a period of lively intestinal activity in a rabbit the hypodermic administration of thirty milligrams of morphine sulphate produced complete cessation of all intrinsic movement within five minutes after a preliminary augmentation. Nicotin, pilocarpin and atropin promptly produced the anticipated results, those produced by pilocarpin being especially striking.

#### REFERENCES

- <sup>1</sup>Van Braam Houckgeest: Pflueger's Arch., 1872, vi, 266.  
<sup>2</sup>Starling and Bayliss: Jour. Physiol., 1899, xxiv, 99; *ibid.*, 1901, xxvi, 125.  
<sup>3</sup>Engelmann: Pflueger's Arch., 1871, iv, 37.

## BARBITAL AS AN ANESTHETIC FOR DOGS\*

BY ARTHUR L. TATUM, M.D., AND ELOISE PARSONS, B.S., CHICAGO, ILL.

IN EXPERIMENTS on dogs lasting throughout a number of hours, investigators have been considerably handicapped on account of deleterious side actions produced by the ordinary anesthetics such as ether or chloretone. Ether itself produces a number of complicating actions and in addition it is impossible to maintain with ether a constant depth of anesthesia. Chloretone is notorious for the prolonged anesthesia obtainable but with the serious objectionable features of progressively lowering blood pressure.

In routine class-room study of the hypnotic series of drugs in our laboratory, it was observed that with a sufficient dosage of barbital the effects of depression to the state of complete surgical anesthesia persisted beyond twenty-four hours. After twenty-four hours the animals were used for acute experiments. The blood pressure was entirely within the normal values of anesthetized animals, while the vasomotor responses were equally as good as those observed during ordinary light ether anesthesia. Pancreatic secretion is as good as that under ether while gastric secretion after secretin and renal secretion from diuretics were far superior to that observed under ether. "Normal" urine secretion during barbital anesthesia appears to be as good as that produced by diuretics under ether anesthesia even in most favorable experiments.

While the pharmacology of "veronal" has been intensively studied by Jacoby and colleagues, we are unaware of the use of barbital in place of other anesthetics. We believe it to be as well adapted for prolonged anesthetics in dogs as is urethane for rabbits. It has a very great advantage over chloretone insofar as barbital does not appear in our experience to produce any fall in blood pressure which is the serious objection to the use of chloretone.

While we make no particular claims for originality in the use of barbital as an anesthetic for dogs, we are not aware of its having been so employed. We feel that the calling attention to this use of barbital may be of considerable service to investigators in carrying out prolonged experiments.

The technic we have employed is as follows: Barbital sufficient to make 0.25 g. per kilo. body weight is dissolved in dilute sodium carbonate solution. This solution administered by stomach tube produces surgical anesthesia in from one half to one hour and lasts for at least eight hours. With a dosage of 0.35 g. per kilo., anesthesia lasts for at least twenty-four hours. By avoiding an excess of sodium carbonate over that required for solution of the barbital, vomiting is not apt to occur. The solid form of barbital may be given equally well in suspension in any suitable vehicle such as acacia or starch solution.

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\*From the Laboratory of Physiological Chemistry and Pharmacology of the University of Chicago.

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## EDITORIALS

### *Variations of Virulence in Tubercle Bacilli*

IN A RECENT address Dr. E. R. Baldwin<sup>1</sup> spoke of attenuation in old cultures of tubercle bacilli, and emphasized our unfortunate lack of any standard procedure whereby to test the relative virulence of different strains.

Variation is one of the most fundamental known laws of biology. All organisms exhibit it to some degree in all their measurable characteristics. Variation among pathogenic microorganisms of the same species has long been a favorite theme of Dr. Theobald Smith's, and recent communications by him,<sup>2</sup> and by Dochez,<sup>3</sup> Topley,<sup>4</sup> and Flexner and Amoss,<sup>5</sup> illustrate our growing knowledge and interest in this fascinating field. The familiar variations in morphology among tubercle bacilli recovered from any large series of cases are an example of the general law.

From this principle alone one could predict with confidence variations of virulence in tubercle bacilli; and conspicuous examples are known, in particular the Saranac Lake R1 strain, to which Dr. Baldwin referred. This



strain originally appeared to be of standard virulence for the guinea pig, but after two or three years of cultivation its pathogenic power decreased greatly and rather rapidly. Now for nearly thirty years guinea pig inoculations with emulsions of this organism have produced with great constancy a disease which is definitely tuberculous, but very benign, and from which the animals recover, with increased resistance to subsequent inoculation with virulent strains.<sup>6, 7</sup> On the other hand, different strains which have been cultivated at Saranac Lake under the same conditions for seventeen and twenty years have not as yet changed appreciably in virulence.<sup>1</sup>

As Krause says, many workers with tubercle bacilli have noticed attenuation under prolonged cultivation,<sup>6</sup> and it has been noted in other circumstances as well. Perez<sup>8</sup> and Manfredi and Frisco,<sup>9</sup> long ago reported the low pathogenicity of bacilli recovered from the lymph nodes of animals inoculated on the surface of the skin and mucous membranes. In our laboratory we have produced relatively mild and chronic disease by inoculating guinea pigs with caseous guinea pig lymph nodes which had been incubated for a few days<sup>10, 11</sup> (and subsequent unpublished work). Cobbett<sup>12</sup> mentions, and many have noticed, the comparatively benign disease caused by inoculation with drying cultures which are not yet entirely dead; and Calmette<sup>13</sup> has reported that marked and rapid reduction of virulence can be effected constantly by the use of bile media.

In some of these cases it is doubtful whether we are dealing with true attenuation or merely with diminished numbers of living bacilli; but in the case of the R1 strain, and of Calmette's bile cultures, there is undoubtedly genuine reduction of virulence, and some of our recent results may have a similar explanation.

Such examples of variation under artificial conditions are of interest, and may be of practical importance in making protective vaccination possible. But it would better help our understanding of the parasite in its relation to the type of disease produced if we could detect variations of virulence in strains freshly isolated from the natural host. On this subject we have remarkably little information, and, as Dr. Baldwin says, not even a reliable method.

The state of our knowledge is admirably set forth in Cobbett's "Causes of Tuberculosis." Briefly, we have the three known races of tubercle bacilli, human, bovine, and avian. On the basis of growth characteristics on artificial media, and of disease produced in guinea pigs, rabbits, cattle, and birds by inoculation with large doses most strains of tubercle bacilli can be quite definitely classified in one of these races, which appear to have a differentiation about as clear as that of Caucasian, Mongolian, and Negro. There is, however, a minority of strains, for example those isolated from lupus, which do not conform exactly to any of these types, and over these strains there is heated controversy, as to whether they are eccentric members of specific races, or transition forms between two races.

Instead of solving this difficulty, study of the data so far available raises new perplexities. Granting that the human type—or the bovine type, which

lends itself best to study—is a distinct race, or subspecies, something fixed, as far as the word is admissible in speaking of living organisms, what about the variations within such a class? If we take only those strains which in the doses commonly employed cause progressive disease in rabbits and cattle, and do not grow vigorously on artificial media—the typical bovine bacilli—how much do they vary in minimum lethal doses for animals of the same breed, age, and weight?

But little work has been done on this problem, which obviously calls for large resources and experiments *en masse*. Two methods suggest themselves: First, the old process of diluting emulsions, the technic of which is described by Calmette;<sup>13</sup> second, the method of Barber, which consists of picking up known counted numbers of bacilli with fine capillary pipettes under the high power of the microscope.<sup>14</sup> The objections to the first method were picturesquely phrased by Cornet in another connection, when he said that it was as inexact to speak of diluting tubercle bacilli in a test tube as to speak of diluting crocodiles in a river.<sup>15</sup> The objections to the second method have been stated by Krause.<sup>16</sup> It is clearly true, as Krause says, that one cannot be certain that all the bacilli picked up are alive, or that they all enter the tissues of the inoculated animal, or that the count is not falsified by several closely cohering bacilli being counted as one. Nevertheless, having made a great deal of use of the Barber technic, we feel sure that for the purpose under discussion it involves less chances of error than the other, or any other method.

In the course of our studies on immunity production by inoculation with living organisms, beginning with one bacillus, in which the Barber method was employed,<sup>17-23</sup> we made a few observations on variation of virulence in cultures, as shown by the minimum number of bacilli which caused visible lesions.<sup>18, 19, 23</sup> The problem should, however, be worked out with many freshly isolated strains from various sources, eliminating, as far as possible, variations developing under the artificial conditions of cultivation.

In such an investigation, any conceivable method involves chances of inaccuracy, which can be minimized by standardizing the technic as rigidly as possible and employing large numbers of experimental animals and controls. When the subject is approached in this way, somewhat as the British Royal Commission approached the problem of types, we shall get some valuable information on spontaneous variations in virulence among tubercle bacilli of the same race. We can then ascertain whether virulence is enhanced by contact with new host material, as seems to be the case with some epidemic diseases, and shall be in a better position to gauge the variations in resistance of different human races and groups.

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<sup>23</sup>Webb: Johns Hopkins Univ. Bulletin, Aug., 1912.

—G. B. W. (C. T. R.)

### *Treatment of Hookworm Infection with Carbon Tetrachlorid*

HALL<sup>1</sup> advocates the use of carbon tetrachlorid in the treatment of hookworm infection. He claims that one dose of .3 c.c. for every kilogram of body weight is sufficient to remove almost all worms. He tested this agent on monkeys, giving as much as five times the dose mentioned above, without producing unfavorable symptoms. He himself took in the morning 3 c.c. of carbon tetrachlorid and did his usual day's work without any inconvenience. The drugs which have been most in favor in the treatment of this disease are thymol, beta-naphthol, and Chenopodium. All of these need to be administered in considerable doses and with caution, since serious results have been known to follow. Carbon tetrachlorid can be prepared cheaply and obtained quite pure. It is a colorless liquid, with a sweetish pungent taste. It is soluble in alcohol and ether and itself dissolves fatty substances. The only medical use to which it has been employed heretofore is as an anesthetic and as an inhalation in hay fever. At one time it was used by the barber in dry shampoo, but fell into disuse because a woman died suddenly after its application. There was some question as to the cause of death.

Niehols and Hampton<sup>2</sup> have taken up this subject and tried it out both upon healthy people and upon those harboring the hookworm. A murderer sentenced to death volunteered to take 6 c.c. This was given one hour after the midday meal and some hours later he passed four ascaris worms. Thirteen days later this dose was repeated in the early morning before breakfast. The patient stated that during the forenoon he felt a little giddy and sleepy, but this soon passed. He was executed a week after he had received the second dose and a postmortem done one hour after death showed no worms in the intestines. Besides, the autopsy showed no lesions which could be attributed to the dose administered. In an agricultural college in Ceylon carbon tetrachlorid was tried out on the students. No purgative was given

<sup>1</sup>Jour. Am. Med. Assn., November 19, 1921.

<sup>2</sup>British Med. Jour., 1922, i, 8.

on the preceding night, all received a dose of 3 c.c. of the drug before breakfast, and no purgative was given after the drug had been administered. The students were instructed to carry on their usual mode of life and no restrictions were placed upon their work, play, or diet. No inconvenience from the drug was recognized, and an average of thirty-six hookworms for each student was recovered from the stools. Next the drug was tried on sixty-four additional students, with like results.

Nichols and Hampton conclude: (1) Carbon tetrachlorid is an efficient anthelmintic; (2) that the drug may be administered safely in doses of 10 to 20 minims to children of three or four years of age; (3) that it aids in the expulsion of *Ascaris lumbricoides* if it is followed by a purgative, but in this respect it is not as effective as *Chenopodium*; (4) that the drug does not seriously deteriorate on keeping; (5) that it is preferable to *Chenopodium* for the following reasons: (a) there is no objection to its taste; (b) it is not necessary to precede or follow its administration by a purge; (c) it is more efficient than *Chenopodium* and is not so distressing; (d) it is much cheaper than any other drug used for this purpose; (e) it can be prepared in a high degree of purity and only chemically pure preparations should be used; (f) the patient is not interrupted in his vocation.

—V. C. V.

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### *The Excretion of Potassium Iodid as a Test of Renal Function*

LINDER,<sup>1</sup> acknowledging that it is unwise to unduly multiply the tests of renal function, thinks that if a new agent proves to be reliable and gives additional information of use in diagnosis, prognosis, or in treatment, it will justify the experimentation. He thinks from his experiments that the iodid test is of great value in prognosis. He has classified his cases according to their iodid output. The patients who excreted less than fifteen per cent of that administered within a given time had the clinical signs of severe renal damage. They showed no tendency to improve; in fact, most of them steadily deteriorated and died in a few months. In the group from which he extracted from fifteen to twenty-five per cent of the iodid, there were two types of cases: (a) Those in whom the power of excretion did not recover and whose subsequent progress was unfavorable, and (b) those in whom the iodid excretion did recover and whose progress was good. All of the last were cases of acute nephritis. Of those who excreted from twenty-five to thirty-five per cent, two recovered the power of iodid elimination and of these one progressed well and one moderately well considering the unfavorable environment to which she returned. The one who failed to improve his iodid excretion showed practically no amelioration of symptoms even while under hospital treatment. The improvement of the iodid output is a valuable sign of recovery. The progress of the patients who eliminated more than thirty-five per cent was good, so far as renal conditions are concerned, but

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<sup>1</sup>Quart. Jour. Med., April, 1922.



unimpaired power of iodid elimination is of less significance than its impairment.

Linder's conclusions are: (1) The iodid test is a reliable indication of nephritis when the excretion falls below thirty-five per cent; (2) in certain cases of nephritis it is the only test of function which shows renal damage; (3) it is a good sign when the excretion remains unimpaired throughout; a fair prognosis when it recovers after being impaired and a bad prognosis and of advanced structural damage when it remains indefinitely in the region of twenty-five per cent. A reduction of the output to fifteen per cent or less is a sign of advanced progressive disease and often foretells death. (4) In certain cases it may be an aid to treatment, indicating a less advanced stage of recovery than can be recognized by clinical signs and other function tests; (5) rarely some iodism is noticed, but in no case was there evidence of a deleterious effect on the kidney.

—V. C. V.

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### *The Relapsing-fever Spirochete of Panama*

S. T. JOHN and Bates<sup>1</sup> demonstrated about a year ago that the relapsing fever in Panama is transmitted by a tick, *Ornithodoros talaje*. They inoculated successfully through the tick, white rats, mice and monkeys. In the paper now under review they have compared the Panama spirochete with those of Obermeier, Novy, Koch, and Dutton. Their conclusions are that the spirochete of relapsing fever in Panama is a distinct species, variety, or strain as compared with the others. Relapsing fever in Panama is probably due to one species, variety, or strain of the relapsing fever spirochete for the present called the Panama spirochete.

—V. C. V.

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### *Typhus Fever in Boston*

SHATTUCK<sup>2</sup> has studied the records of the Boston City Hospital for the past ten years and arrives at the conclusion that the typhus problem in that City has not been significant from the diagnostic standpoint. However, he thinks that the potential epidemiologic significance of typhus, especially of atypical cases, requires that all suspected cases be treated with the greatest care in order to arrive at a correct diagnosis. Under possible cases, he includes a considerable group having eruptions or temperature curves suggestive of typhus. In this group there are two classes: First, those undiagnosed, and second, those in which diagnosis is not supported by exact facts. Careful investigation of such cases would perhaps show that typhus fever is in reality more common in Boston than it appears to have been in recent years. The Widal reaction is commonly present in typhus fever. Therefore,

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<sup>1</sup>Am. Jour. Trop. Med., 1922, ii, 251.

<sup>2</sup>Am. Jour. Tropical Med., 1922, ii, 225.

it is not *per se* an obstacle to the diagnosis of this disease. The proteus reaction of Wilson, Weil and Felix is not infallible, but is a valuable aid. In order not to be misled by this reaction it is necessary that it should be adequately controlled. From time to time the test should be checked with known typhus serum. In the interpretation of the results of this test all known facts regarding it should be borne in mind and clinical evidence should be considered. Shattuck thinks that the surest means of diagnosing typhus fever during life is by histologic examination of excised bits of skin. Pathologic changes found in the guinea pig after inoculation with material containing the virus are believed to be constant, but are sometimes difficult to demonstrate. Inoculation of guinea pigs may be used as an aid in diagnosis. Of great importance for diagnosis is the almost constant absence of leucopenia in typhus and its nearly constant presence in typhoid. There is an increase in number of cells in the cerebrospinal fluid, but this may be slight or marked. Failure to recognize this may lead to erroneous interpretations. Wiener's color reaction is easily made and warrants further trial.

—V. C. V.

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### *A Death Caused by Ascarides*

CALDWELL<sup>1</sup> reports that in the village of El Pilon in the Province of Panama in 1921 a baby girl, twenty-two months of age, died under the following conditions: She had been given at 6 A.M. one-half minim of the oil of *Chenopodium* and this was followed two hours later by Epsom salt. There being no movement of the bowels and no disagreeable symptoms appearing, after two hours more a saline enema was employed. This was followed by evacuation of innumerable ascarides. About 5 P.M. the child became very weak and ascarides in bunches came from her mouth and nose, collected in her throat, blocked the larynx, and caused death from failure of respiration. Ten hours after death, ascarides were still coming from her mouth.

This quite equals the stories found in some of the older reports of the prevalence of this parasite in the Tropics.

—V. C. V.

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### *War Nephritis*

DYKE<sup>2</sup> has made a study of the remote effects of this disease. It will be remembered that during the War the men in the trenches were inclined to develop evidences of nephritis, characterized by albuminuria, hematuria, and edema. Some guesses were made at that time as to what the ultimate result would be. Dyke has taken one hundred typical cases of war nephritis, and with the aid of the records of the Pension Department he has followed

<sup>1</sup>Am. Jour. Trop. Med., 1922, ii, 213.

<sup>2</sup>Quart. Jour. Med., April, 1922.

up their histories to the middle of 1921,—a period of about four and one-half years from the onset of the disease. Of the one hundred cases it was found that three had died; one aged thirty-seven; one, twenty-six; one, twenty-nine. One of these died of scarlet fever, and it is a question whether the nephritis had anything to do with the fatality of the scarlet fever. The cause of death in the remaining two cases was given as nephritis, and it seems that in these instances the war nephritis became chronic and led to a fatal termination in eighteen and twenty-seven months respectively. Of the ninety-seven remaining cases, sixty-five were eventually returned to duty, but of these, two had recurrences while serving in France and finally became pensioners on account of this condition. The remaining thirty-two were discharged as unfit.

Dyke's conclusions are stated as follows: (1) Seventy per cent of cases of war nephritis at all ages terminated in recovery before the lapse of one year; (2) the proportion of cases so terminating is slightly higher under than above the age of thirty-five; (3) cases not fully restored to health by the end of twelve months became chronic; (4) the occurrence of symptoms of uremia at the outset has no unfavorable significance as regards the later prognosis; (5) in cases becoming chronic there is a tendency for fatal issue to be determined by circulatory or respiratory conditions; (6) the proportion of deaths from pulmonary tuberculosis among patients in the chronic stage is high.

Dyke calls attention to the fact that early in the War, Osler cautioned against giving too favorable a prognosis. Abercrombie has reported on the after-histories of 171 cases for periods from twenty-four to thirty-two months from onset. His observed mortality at all ages for that period is three per cent; the same as that observed by Dyke.

—V. C. V.

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## *ORIGINAL ARTICLES*

### THE TECHNIC OF BASAL METABOLIC RATE DETERMINATION IN PSYCHONEUROTIC PATIENTS\*

BY B. S. LEVINE, PH.D.,† WAUKESHA, WIS.

IN MAKING basal metabolic rate determinations of psychoneurotic persons, several technical points must be observed, in order that more efficient results may be obtained. The general appearance of the apparatus, for instance, should present as simple an arrangement as possible, so that it may arouse no suspicion in the mind of the patient that he is to be subjected to a complicated procedure. It is best that the pipes and most of the connections be concealed from view, and that the bed upon which the patient is to rest appear but little different from the hospital cot to which he is accustomed.

To attain this end, the following arrangement has been adopted at this hospital in connection with the Tissot-Haldane combination. An ordinary hospital folding cot is used as the bed. It is placed in the corner of a quiet room with the foot end close to a window. The intake air pipe, *A* (Fig. 1), is fastened to the bed under the spring in a way which completely conceals it from view. At the foot end, the pipe is directed vertically upward for about 5 to 7 inches by means of an elbow, and by use of another elbow it is extended out horizontally for about 10 to 12 inches. This horizontal extension, *A* (Fig. 2), passes through a hole in a board about seven inches wide, placed in the window in a manner similar to that of a window screen. The lower part of the window rests directly over the top edge of the board sufficiently tight to prevent the wind from blowing in at the junction. If necessary, a strip of felt may be nailed at the junction in the winter.

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The pipe passes through the board as near the window sill as possible, and is completely concealed from view when the bed is made up.

The intake air pipe is directed upward at the head end of the bed also by the use of an elbow, just sufficiently to enable the fastening over it of a flexible ribbed rubber hose. This projection, *B* (Fig. 1), is tapered at the end, so that the hose may be attached to it with facility. At the other side of the head end of the cot is fastened an arrangement which connects the spirometer to the "Expiration Hose." Its construction is shown in Fig. 3. *A* is a T-shaped connection, *B* are the horizontal short arms of the *T* made of short pipe-pieces tightly screwed into the *T* and tapered at the ends, to facilitate placing the hose connections over them. *C* is the vertical long arm made of a pipe 24 inches long, screwed into the *T*, and which is used merely as a holder. The top opening of *C* is tightly plugged before it is connected with the *T*, to prevent the escape of the expired air. The entire combination is fastened by means of its long arm to the head-end of the cot, opposite the

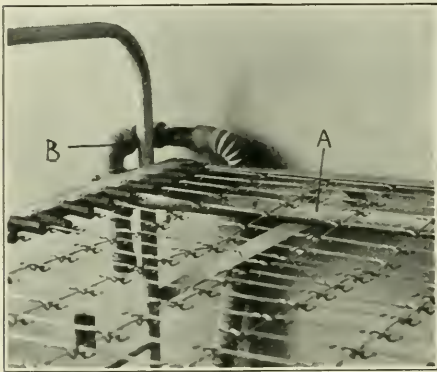


Fig. 1.



Fig. 2.

intake connection, just high enough to allow slipping over it the flexible ribbed "Expiration Hose," *D*, yet low enough to conceal it when the bed is made up.

The device used for the control of the inspiration and expiration is a point of next importance. The gas mask, as described by Boothby and Sandiford,<sup>1</sup> has been in use for that purpose at this hospital for several months, and as a result, the following objections were brought out. Nearly all the patients at the hospital had some experience with gas masks, and many of those tested manifested a definite reaction, due to the associations brought about by the mask, affecting the end-result markedly. Patients possessed of psychopathic tendencies and lacking the natural intelligence necessary for the proper cooperation in the carrying out the B.M.R. determination procedure, could not keep still when the mask was fastened over their faces. Indeed, they were invariably so restless that the mask shifted, causing considerable leakage, and resulting in such variations in the final rates, that it was difficult to come to a conclusion as to which was the

<sup>1</sup>Laboratory Manual of the Technic of B. M. R. Determinations, W. B. Saunders Co., Philadelphia.

normal rate of the patient. In one case, where the patient voluntarily came for rate determinations six times, no two rates obtained were sufficiently close to be called duplicates.

Many of the confirmed hysterics and of the anxiety patients behaved much the same as did the psychopaths for the first two or three tests, but finally became accustomed to the procedure, though they never expressed a liking for it.

Finally, in cases with certain facial deformities, caused by wounds while in action, proper adjustment of the mask was impossible, in spite of all its flexibility. The use of the mask had to be abolished, and the mouth piece and nose clip were tried out. About fifty patients, previously tested by the use of the gas mask, were retested by the mouth piece and nose clip method, and all but one expressed the unsolicited preference for the latter. The one differing in the opinion was a woman, (army nurse), who had a

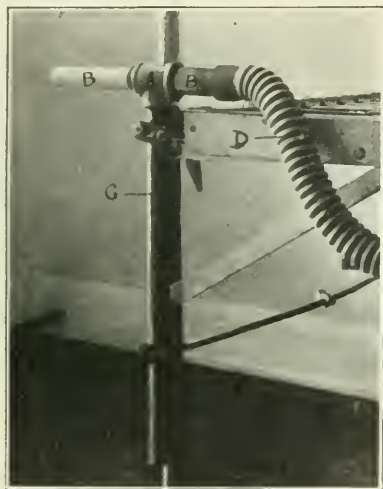


Fig. 3.

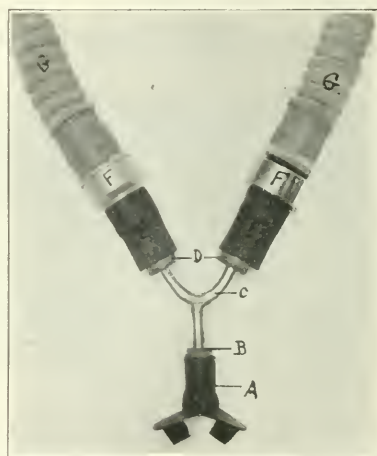


Fig. 4.

distinct thyroid involvement with a consistent rate of plus 57 per cent. This resulted in the exclusive adoption of the mouth piece and nose clip, although masks are had in store to accommodate a particular necessity which may arise.

The arrangement now in use is shown in Fig. 4. *A* is the mouth piece, *B* is a No. 1 rubber stopper, *C* is a nickel-plated metallic Y-shaped tube, *D* are No. 5 rubber stoppers, *E* are short pieces of gardener's rubber hose. *F* are the controlling air valves, and *G* the flexible ribbed rubber hose.

In carrying out a determination on new cases where it is suspected an objection or a lack of proper cooperation may be encountered, the following procedure is resorted to, which may be described as consisting of three steps.

1. With the mouth piece, Y-shaped metallic tube and the rubber stoppers detached from the rest of the arrangements and with the ribbed rubber hose and valves connected to the intake and expiration pipes, but properly concealed from view, the patient is placed on the bed, and his temperature,

respiration rate, and blood pressure are taken. These are procedures to which he has been subjected before, and almost invariably induce the patient to display a certain amount of confidence and cooperation. After the required resting period has elapsed, the mouth piece is introduced, and is properly propped up by a small pillow as is shown in Fig. 5, and the patient is allowed to rest in this position for a minute or two.

2. The nose clip is fastened over the nose, as is shown in Fig. 5, and the patient is allowed time to adjust his respiration rhythm.

3. The flexible ribbed rubber connections are attached to the corresponding ends of the Y-shaped tube, as is shown in Fig. 6, and the determination is completed in the usual manner.

Where proper cooperation on the part of the patient is expected, or when a repeat determination is carried on, the mouth piece is connected to the ribbed hose while the patient is resting, and the attached mouth piece introduced into the mouth, the nose clip fastened, and the determination proceeded with at once.

It is the custom at this institution to carry out duplicate determinations



Fig. 5.



Fig. 6.

in succession, to rule out possibilities of errors in the procedure. To this end, the nose clip alone is usually removed at the completion of the first determination, and the patient is allowed to breathe through the nose, but with the mouth piece in the mouth, while the readings and the temperature of the spirometer are taken and the samplers are filled. As soon as this is done, and the spirometer is emptied, the nose clip is replaced, and the duplicate determination proceeded with at once.

This was found to save considerable time and annoyance to both the laboratorian and to the patient. In individual cases, the mouth piece as well may be removed, if such a procedure is indicated, but the writer has found only two out of fifty cases in which such a procedure seemed wise, though not necessary.

At the end of a duplicate determination the mouth piece is detached, washed with soap and hot water, sterilized in alcohol or carbolie acid, again thoroughly rinsed with hot water, and used as before. This can be done while the patient is resting; or two mouth pieces may be had, so that one may be washed and sterilized, while the other one is used.

As stated above, the Tissot-Haldane combination has been in use at this

institution, although other combinations, considerably simpler in appearance and in manipulation are now obtainable. It should be stated, however, that the Tissot-Haldane principles yield results which may be designated as dependable even for scientific purposes. The additional data, such as the variations in the Respiratory Quotient variations in the ventilation, and in the nature of the respiration, which the Tissot-Haldane combination affords the investigator, have been found highly useful in differentiating types of neurotic patients, even when their rates stayed within the normal limits. If properly observed and properly interpreted, this procedure of Basal Metabolic Rate Determination more than compensates the diagnostician for the extra work it demands.

Grateful acknowledgment is made to Surgeon Lawrence Kolb, Medical Officer in Charge of this Hospital, for his permission to prepare and publish this paper; to P. A. Surgeon (R) C. Covey, Clinical Director, for his general assistance, and to Mr. D. E. Roberts of this Hospital for preparing the photographs reproduced herein.



## COMPARATIVE CONCENTRATION OF UREA IN THE BLOOD AND SALIVA IN A SERIES OF PATHOLOGIC CASES\*

BY HERBERT W. SCHMITZ, M.D.,† NEW YORK CITY

IT HAS long been established that urea diffuses very readily through cell membranes. Red blood cells placed in a urea solution almost instantly acquire the same concentration of urea as that of the solution itself, without any noticeable injurious effect upon the cells themselves.<sup>1</sup> The work of Marshall and Davis<sup>2</sup> has shown that urea is found in all tissues and body fluids in approximately uniform concentration. This uniform distribution also occurs when the urea content of the body is increased, either by intravenous injections of urea, or by defective elimination of this substance, or by both. Cullen and Ellis,<sup>3</sup> and Myers and Fine<sup>4</sup> have pointed out the remarkably close relationship between the concentration of urea in the blood and spinal fluid. As a result of these observations it appeared of interest to study the relationship between the concentration of urea in the blood and saliva in various pathologic conditions.

Urea has been regarded as a constituent of normal saliva for many years. Rabuteau<sup>5</sup> in 1873 observed that after taking 5 grams of urea and 200 c.c. of water, the salivary secretion was markedly increased, and that the saliva had a peculiar taste. It immediately occurred to him that this peculiar taste might be due to urea secreted in the saliva. This fact he verified by chemical analysis, 35 c.c. of saliva being found to contain 0.0235 grams of urea. An analysis of normal saliva made at this time also showed the presence of this compound. Rabuteau, however, was not the first to detect urea in saliva. Samuel Wright<sup>6</sup> in 1841 reported a "Case of Ascites in Which During a Spontaneous Ptyalism That Occurred after Tapping, Urea Was Detected in the Saliva." The secretion was chocolate in color, slightly ammoniacal in odor, and contained 130 mg. of urea per 100 c.c. On two previous occasions Wright demonstrated urea in the saliva of dogs poisoned by corrosive sublimate. Von Zerschwitz<sup>7</sup> in 1883 studied the urea content of saliva in normal persons and in cases of nephritis. He concluded that the determination of urea in saliva in normal individuals was practically impossible, even with their most delicate methods since only occasionally traces of this compound could be detected with the amyl-alcohol method; but that in cases of nephritis, its detection in saliva was comparatively simple and accurate, and that the urea curve varied in direct proportion to the intensity of the kidney lesion. How-

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Possibilities of the utilization of the determination of the concentration of the urea in the saliva was simultaneously discussed by Drs. Rowntree and Myers at the Seventy-Third Meeting of The American Medical Association, June, 1922 (Jour. Am. Med. Assn., 1922, lxxix, 1389-1390). Since that time and after our paper was submitted for publication, Hench and Aldrich in Dr. Rowntree's Department at the Mayo Clinic have given a brief discussion of this subject (Jour. Am. Med. Assn., 1922, lxxix, 1409).

†Medical Fellow of the National Research Council.

ever, no attempt was made to express the urea content in terms of grams or milligrams, the terms, traces, small and large amounts of urea crystals, being employed. Since the methods used by these early investigators were of questionable accuracy, their results must be taken with reserve.

Marshall and Davis, in their analysis of the urea distribution in the various tissues and body fluids, found that the urea concentration of the parotid gland was exactly the same as that of the blood, although they reported no urea determinations on the mixed saliva.

Some of our determinations (made by Dr. Myers) were begun during the early part of 1922. The observations include the urea content of both blood and mixed saliva and ammonia in saliva. The blood and saliva specimens were obtained the same day, except as indicated in the table. The estimation of urea was performed by the method described by Myers.<sup>8</sup> In case of the saliva, 2 c.c. of the filtered secretion were used for the analysis. As we shall see later, the urea in the saliva as determined by this method, represents the combined value of both urea and ammonia. The ammonia was determined by the same method, omitting the addition of urease and incubation.

#### METHOD OF COLLECTING SALIVA

The teeth are carefully brushed, and the mouth thoroughly rinsed out with water. As a salivary stimulant, the patient is given a small piece of paraffin to chew, and all the saliva secreted is collected in a small clean cup to which 2-3 drops of molecular phosphate have been added. The determinations are made immediately after the specimens have been collected.

#### DISCUSSION

At the time this work was begun, the saliva was also analyzed for ammonia, and it was found to contain variable amounts of this compound. The amount of ammonia present did not seem to bear any definite relation to the combined amount of urea and ammonia, for in some cases it almost equaled, but in others only represented a small fraction of the total amount of urea and ammonia. Any relationship between the ammonia concentration of the blood and saliva would appear extremely improbable, since the concentration in the blood is so much lower and so much more constant.<sup>9</sup>

TABLE I

AMOUNT OF AMMONIA IN SALIVA AFTER TEETH AND MOUTH HAD BEEN THOROUGHLY CLEANED

DETERMINATION	UREA PLUS AMMONIA N	AMMONIA N
	MG. PER 100 C.C.	
1	11.1	6.5
2	5.4	4.4
3	9.8	4.6
4	8.2	4.3
5	73.5	11.5
6	67.7	7.6
7	111.9	5.3

Possible sources of ammonia are: (1) From the saliva, secreted as a normal constituent. (2) From putrefaction of food particles present between the teeth. (3) From conversion of urea to ammonia due to bacterial activity.

That ammonia is not a normal constituent of parotid saliva is evidenced by the fact that specimens of parotid saliva obtained by means of a cannula inserted into Stenson's duct did not show the presence of this compound. Specimens of saliva collected after the teeth had been carefully brushed and the mouth thoroughly rinsed were analyzed immediately for ammonia, and were found to contain small amounts of this substance in every instance (Table I). The probability of the ammonia being due to putrefaction of food particles is practically removed by this test. To determine whether or not the ammonia was derived from urea, specimens of saliva were analyzed immediately for urea and ammonia and again after standing for several hours. As shown in Table II, it is evident that the combined urea and ammonia values decreased and the ammonia values increased. The increase in ammonia must have taken place at the expense of the urea, for the latter compound was considerably reduced in amount. When 2-3 drops of molecular phosphate were added, there was no change in the combined value of urea and ammonia, but the ammonia had increased considerably and the urea decreased proportionately, supporting the assumption that the urea is the source of ammonia in mixed saliva (Table II).

TABLE II  
SALIVA ANALYZED IMMEDIATELY FOR UREA AND AMMONIA AND AFTER STANDING SEVERAL HOURS

SPECIMEN	ANALYZED IMMEDIATELY		ANALYZED AFTER 8 HRS.		ANALYZED AFTER 24 HRS.	
	UREA PLUS $\text{NH}_3\text{-N}$	$\text{NH}_3\text{-N}$	UREA PLUS $\text{NH}_3\text{-N}$	$\text{NH}_3\text{-N}$	UREA PLUS $\text{NH}_3\text{-N}$	$\text{NH}_3\text{-N}$
	MG. PER 100 C.C.					
1	73.5	11.5	65.8	22.0	40.0	17.0
2	83.1	11.8			74.2	24.7
*3	67.0	7.4			65.7	17.6
*4	26.3	14.9			26.7	25.6
†5	28.8	5.0			28.2	4.2

\*Molecular phosphate added to saliva.

†Saliva run through Berkefeld filter.

A specimen of saliva to which molecular phosphate had been added was run through a Berkefeld filter and collected in a sterile flask. The filtered saliva was immediately analyzed for urea and ammonia, and again after twenty-four hours. Both determinations gave practically the same results (Table II), thus suggesting the possibility that the greater part of the ammonia content of saliva is derived from urea, the result of bacterial activity and not as the result of an enzyme.

Table III presents forty-five simultaneous determinations of urea in the blood and saliva in various pathologic conditions. The observations are listed according to the amount of urea retention in the blood. It will be noted that in the majority of cases, the urea content of the saliva runs somewhat lower than that of the blood, but that as the urea concentration of the blood is increased, the urea value of the saliva likewise rises. In the forty-five determinations, the average salivary urea content amounts to 89.4 per cent of that of the blood. Myers and Fine<sup>4</sup> found that the concentration of urea in spinal fluid averaged 88 per cent of that of the blood.

TABLE III  
COMPARATIVE CONCENTRATION OF UREA IN THE BLOOD AND SALIVA

CASE	AGE	SEX	BLOOD	SALIVA		REMARKS
			UREA N	UREA PLUS	AMMONIA N	
				AMMONIA N		
				MG. PER 100 C.C.		
20916	47	M	10.7	11.2		
21707	52	M	10.7	8.8	6.5	Chronic Cholecystitis, Cholelithiasis.
21676	34	M	12.1	10.8	10.3	Ventral Hernia. Intraabdom- inal Adhesions.
21001	52	M	15.2	16.1	....	Cerebro-Spinal Lues. Chronic Plumbism. Mitral Regurgitation. Hypertension.
21766	53	M	15.0	13.0	9.7	Chronic Appendicitis with Adhesions.
21702	44	F	15.3	19.5	....	Diabetes Mellitus.
21806	48	M	15.4	12.5	8.6	Carcinoma of the Esophagus.
21211	34	M	15.6	11.5	....	Hyperthyroidism.
21935	20	M	15.9	10.0	4.7	Psycho-neurosis.
21975	48	M	16.0	17.5	....	Chronic Cholecystitis.
21836	34	F	16.5	10.1	5.0	Psychoneurosis. Obesity.
21975	48	M	16.5	17.2	12.5	Chronic Cholecystitis.
21975	48	M	16.5	17.7	....	Chronic Cholecystitis.
21639	46	F	17.5	14.8	7.3	Nephrosis. Chronic Myocarditis.
21771	53	M	18.1	10.5	8.5	Prostatic Obstruction. Vesical Calculus.
21229	60	M	18.2	14.5	....	Lues.
20438	45	M	18.7	22.5	17.7	Chronic Nephritis.
22170	70	M	20.2	17.8	13.1	Diabetes Mellitus. Diabetes Mellitus.
20760	54	M	20.2	18.7	16.9	Edematous Prostate.
22170	70	M	21.7	20.6	....	Chronic Nephritis. Myocardial Insufficiency.
21008	49	M	21.8	18.3	....	Diabetes Mellitus.
20071	60	M	22.3	20.6	11.6	Edematous Prostate.
15526	31	M	22.6	17.0	10.0	Prostatic Hypertrophy. Chronic Parenchymatous Nephritis.
21864	45	M	23.0	19.7	17.7	Diabetes Mellitus.
20438	45	M	23.2	20.2	10.0	Cerebrospinal Lues. Diabetes Mellitus.
22088	53	M	25.6	31.1	....	Chronic Nephritis.
21498	62	M	30.7	25.1	....	Essential Hypertension.
20071	60	M	30.9	22.7	15.9	Prostatic Obstruction.
20071	60	M	34.8	26.2	14.9	Prostatic Hypertrophy.
21240	47	M	35.8	27.5	....	Prostatic Hypertrophy. Chronic Interstitial Nephritis.
21498	62	M	37.4	37.1	....	Nephrolithiasis.
21498	62	M	39.7	42.1	....	Prostatic Obstruction.
20071	60	M	42.8	28.5	....	Prostatic Obstruction.
21498	62	M	46.8	43.5	....	Prostatic Hypertrophy.
16515	55	M	60.0	48.0	20.0	Prostatic Obstruction. Arteriosclerotic Kidneys.
19412	26	M	61.4	45.5	....	Hypertension. Chronic Interstitial Nephritis.



TABLE III—CONT'D

COMPARATIVE CONCENTRATION OF UREA IN THE BLOOD AND SALIVA

CASE	AGE	SEX	BLOOD	SALIVA		REMARKS
			UREA N	UREA PLUS	AMMONIA N	
				AMMONIA N		
				MG. PER 100 C.C.		
*17135	55	M	61.5	40.0	30.0	Arteriosclerotic Kidneys. Hypertension. Cardiac Hypertrophy. Dilated Aorta.
19412	26	M	62.5	55.5	23.1	Chronic Interstitial Nephritis.
19271	34	M	64.1	43.6	...	Chronic Nephritis. Chronic Secondary Anemia.
*17177	53	M	65.0	79.0	10.0	Polycystic Kidneys. Myocardial Insufficiency.
*16753	50	M	74.9	83.0	60.0	Chronic Interstitial Nephritis. Cardiac Hypertrophy and Dilatation.
19412	26	M	75.0	50.0	...	Chronic Interstitial Nephritis.
19412	26	M	86.2	73.5	11.5	Chronic Interstitial Nephritis.
19412	26	M	98.0	98.7	...	Chronic Interstitial Nephritis.
22065	65	F	100.0	111.9	5.3	Chronic Interstitial Nephritis.

\*Specimens not collected on the same day.

Since the saliva acquires practically the same urea concentration as the blood, salivary urea determinations will, therefore, give us definite information in pathologic cases with urea retention. In cases where it is impossible or impracticable to obtain specimens of blood, a determination of the urea concentration of the saliva will serve as a fair index of the functional activity of the kidneys.

## SUMMARY

Comparative simultaneous analyses on forty-five specimens of blood and saliva, representing various pathologic conditions, are reported. The urea content of saliva averaged 89.4 per cent of that of the blood. Salivary urea determinations may be employed in determining the functional activity of the kidneys where for any reason it is impracticable to obtain blood specimens.

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- <sup>7</sup>von Zerschwitz: Inaugural Dissertation, Erlangen, 1883.
- <sup>8</sup>Myers: Practical Chemical Analysis of Blood, The C. V. Mosby Co., St. Louis, 1921, pp. 34-35.
- <sup>9</sup>Nash and Benedict: Jour. Biol. Chem., 1921, xlvi, 463.

## A REPORT OF SEVEN CASES OF NICOTINE POISONING\*

By WM. D. McNALLY,† A.B., M.D., CHICAGO, ILL.

POISONING by the alkaloid nicotine is increasing, due to its more frequent use as an insecticide in recent years. The commercial preparations containing from 8 to 43 per cent of the alkaloid nicotine are used in very dilute solutions as insecticides. The free alkaloid is a colorless oily liquid which rapidly becomes brown closely resembling whisky in appearance which accounts for its accidental consumption.

In a previous paper (*Jour. Lab. and Clin. Med.*, 1920, iv, No. 4) I reported five cases of poisoning by this alkaloid. Since that time material has been submitted from seven other deaths; viz., organs in four instances and bottles of insecticides in the three others. There were verdicts, "suicide" in four, "accident" in two, and in one "undetermined." Five of these people were found dead, in the remaining two it is known that death occurred in less than five minutes. A brief history of each follows:

No. 1, J. A. Nov. 6, 1919, took  $\frac{1}{2}$  oz. of an insecticide, which upon analysis contained 40.86 per cent nicotine. After taking the poison he walked up a flight of stairs from the basement of his home to the kitchen, where he vomited; he then walked about twenty feet to his bedroom and dropped dead. The time estimated by his family was less than five minutes after he started to walk from the basement until death occurred. There was no autopsy. The Coroner's verdict read "took nicotine with suicidal intent while temporarily insane."

No. 2, E. H. P. Sept. 22, 1919, (Footnote, The newspaper publicity given this case may have been responsible for other poisonings by nicotine, as was previously noted in deaths by mercuric chloride, McNally, W. D., *Med. Rec.*, Feb. 1, 1919). A camouflaged breakfast and robbery had been staged to give the appearance of murder. The deceased was found gagged and bound in a large armchair in the kitchen of his home. Upon the handkerchief gag and upon his shirt were brown stains; on the floor there was a broken tumbler. The ropes binding the arms were not securely tied on the left arm, and the right arm had  $9\frac{1}{2}$  inches of slack, which was sufficient to permit a person to slip down in the chair and drink from a glass tumbler. The postmortem examination of the body demonstrated a hyperemia of all the organs, otherwise nothing grossly was observed, except a peculiar odor in the stomach. A chemical examination of the organs revealed the presence of nicotine. (See Table for amounts). Nicotine was found on the gag and bosom of shirt. In the coffee and milk on the table tests failed to show the presence of nicotine. The brain contained none, the kidneys and liver gave only traces. The authorities were undetermined as to whether this was a suicide or murder.

No. 3, D. D., (male), March 11, 1920, had been drinking heavily and took 1 oz. of an insecticide containing nicotine. No autopsy was held. The verdict of the Coroner's jury was given as "suicide while despondent and temporarily insane." An empty 1 oz. bottle which had contained nicotine was submitted.

No. 4, G. S., May 7, 1920, is said to have taken nicotine by mistake for cascara and

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†Chemist to the Coroner of Cook County, Chicago, Ill.

TABLE NO. I.  
NICOTINE IN MILLIGRAMS

LAB. NO.	STOMACH		STOMACH CONTENT		STOMACH AND CONTENT		BOWEL		LIVER		KIDNEY		URINE		BRAIN	NICOTINE BOTTLE LIQUID %
	In total weight material rec'd.	In 100 grams	In total weight material rec'd.	In 100 grams	In total weight material rec'd.	In 100 grams	In total weight material rec'd.	In 100 grams	In total weight material rec'd.	In 100 grams	In total weight material rec'd.	In 100 grams	In total weight material rec'd.	In 100 grams		
E. H. P. Undetermined	5173															
		In 138 G. 990.50 mg.	In 717.60 mg.				In 807 G. 782.70 mg.	In 97.00 mg.	Trace		Trace				Negative	
G. S. Accident	6108															
		In 224 G. 441.79 mg.	In 200.70 mg.	In 310 cc. 3184.2 mg.	In 1271.7 mg.											
M. M. Suicide	6387															
				In 282 G. 1610.2 mg.	In 571.0 mg.	In 276 G. 9.6 mg.	In 3.5 mg.	In 173 G. 9.1 mg.	In 5.3 mg.	In 135 G. 14.4 mg.	In 10.7 mg.	In 280 cc. 1.1 mg.	In 0.4 mg.			20.06 %
M. V.	7019															
		In 161 G. 18.5 mg.	In 11.4 mg.			In 478 G. 65.1 mg.	In 13.6 mg.	In 1255 G. 37.0 mg.	In 2.9 mg.	In 372 G. 16.1 mg.	In 4.6 mg.					

was found dead in bed with no glass or bottle near. The postmortem demonstrated a hyperemia of all the organs as already noted. A chemical examination of the organs revealed that death was due to nicotine poisoning. At the inquest a bottle of nicotine was produced which was said to have been found in the clothes hamper in the basement. The results of the chemical examination can be seen in the Table.

No. 5, M. M., July 19, 1920, a housemaid took an unknown quantity of a bottle labeled "Nikoteen" containing 28.60 per cent nicotine. She had been drinking heavily and when informed that her services as housemaid were no longer required, took the poison and was found dead the next morning. A postmortem examination was made and the organs submitted for chemical examination.

A verdict of "suicide while temporarily insane" was rendered. Investigation revealed the fact that the deceased had been confined in an asylum for four months the year prior to her death.

No. 6, F. R. D., Sept. 12, 1919, a boy seven years of age, was given one-half a dram of an insecticide in mistake for a cough mixture. The bottles were practically of the same design, both containing dark brown liquid. The child walked 18 feet after taking the liquid, death occurring in less than five minutes. There were no organs submitted. A chemical examination of the insecticide gave 21.55 per cent nicotine.

No. 7, M. V., Los Angeles, was found dead the morning of April 5, 1921 and the body was shipped to Chicago. The mysterious circumstances surrounding this death caused exhumation of the girl's body, April 10, 1921. The death certificate gave the cause of death as "diphtheria," evidence of which could not be seen by the pathologist here at the autopsy. The postmortem showed that an abortion had been recently performed. A chemical examination of the organs revealed that death was due to nicotine poisoning.

In all of the postmortem examinations mentioned, no disease from natural processes was found which in any way contributed to death.

During a toxicologic investigation of several thousand deaths, it has been noted that in certain embalmed bodies that the alkaline distillate from the steam distillation of the tissues gives several of the reactions for nicotine. The results of the research upon the alkaline distillate will be the subject of a subsequent paper in preparation.



## THE LOCAL WASSERMANN IN THE EARLY DIAGNOSIS OF PRIMARY SYPHILIS\*

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### INTRODUCTION

THE POSITIVE diagnosis of syphilis in the early primary stage of the disease, before the occurrence of systemic invasion, is of the utmost importance in prognosis and treatment. But it is in the primary stage that an absolute diagnosis is sometimes most difficult. In the early stages, the blood Wassermann reaction is usually negative. The presence of *Spirochete pallida* in the lesion is the only positive criterion of a primary chancre. Occasionally, no dark-field microscope is available. A certain percentage of cases which later develop positive blood Wassermanns, fail to show spirochetes in the lesion on repeated careful examinations. Lastly, lesions which have been treated locally with a spirocheticide, usually show no spirochetes. Consequently, because of both the great importance of an early diagnosis, and the absence of any single infallible method, an additional means of diagnosing a primary chancre is of inestimable value. In certain cases, the "local" Wassermann reaction, performed on the surface serum of a suspected primary chancre, appears the only means of making a positive diagnosis of syphilis, at the time when a clear-cut diagnosis is of greatest value.

Within the past year, Klauder and Kolmer<sup>1</sup> have reported the results of studies on the Wassermann reaction carried out on various body secretions, as well as transudates and exudates, particularly with reference to the site of origin of the specific complement-fixing antibodies. To demonstrate the origin of specific complement-fixing antibodies locally as well as hematogenously, these workers studied the Wassermann reaction of the surface fluid from a number of primary chancres. These tests "yielded almost uniformly a four-plus reaction. In some instances, positive results were obtained before the test appeared positive in the blood, which apparently excludes the possibility of the positive reaction in the chancre fluid being due to admixed blood." Since the completion of our present study, Klauder and Kolmer<sup>2</sup> have published the results of the local Wassermann on 14 cases, and have elaborated the suggestion contained in their first paper of the practical value of the local Wassermann as an aid in the early diagnosis of syphilis. Their results will be discussed in comparison with our own findings below.

### MATERIAL AND METHODS

*Material.*—Most of the cases in this series were referred for study through the courtesy of the members of the Department of Dermatology and Syphilis

\*From the Department of Medicine and the Division of Dermatology and Syphilis of the Medical School, University of Minnesota, and the Division of Venereal Diseases, Minnesota State Board of Health.

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of the Medical School, the remainder coming to the State Board Laboratory from various physicians for dark-field examinations. Although only cases in which the diagnosis of primary chancre appeared probable were studied, every case in which the local Wassermann test was performed, is included, so that the remarkably constant results are not the distorted effect of omitting any negative reactions. The presence of *Spirochete pallida* is taken as the only unquestionable criterion of a true primary chancre. The usual clinical data, including the time from exposure to the appearance of the lesion, the time since the appearance of the lesion, the presence of satellite or general adenopathy, the occurrence of secondary lesions, and the blood Wassermann at the time of examination are included to complete the clinical picture.

*Technic.*—The surface of the chancre is sponged off with normal saline, after which it is dried and gently squeezed. Ordinarily, sufficient serum exudes without difficulty. If there is not enough serum present, the surface of the lesion is scratched lightly with the end of a glass capillary tube, since the admixture of a little blood in no wise interferes with the reaction. The surface serum is collected by capillary suction in a fine glass tube, graduated to 0.1 c.c. From one to two tenths of a cubic centimeter of serum are usually obtainable. A 0.1 c.c. drop of the serum was used in all cases. Dilutions of 1 to 8, 1 to 16 and 1 to 24, giving serum in amounts of 0.0125, 0.00625 and 0.004 c.c., respectively, were made up to the extent of the serum obtained. The routine Wassermann technic used by the laboratory of the Venereal Division of the Minnesota State Board of Health, which during a corresponding length of time was used in about 49,000 blood Wassermann tests, was employed in all cases. This is the standard antisheep cell hemolytic system, using a single, noncholesterinized, alcoholic extract of human heart for antigen. Controls for anticomplementary action of the chancre serum were negative in all cases.

#### RESULTS

*Blood Wassermans.*—Thirty-four cases of demonstrated primary chancre and two with proved nonluetic lesions are included in this series. The clinical and laboratory findings are tabulated in Table I, and summarized in Table II. For convenience, the cases are divided into four groups: Group I: Ten cases with positive dark-field, positive blood Wassermann and positive local Wassermann. Group II: One case with positive dark-field, positive local Wassermann and weakly positive blood Wassermann. Group III: Twenty-three cases with positive dark-field, positive local Wassermans but negative blood Wassermans. Group IV: Two cases with all the laboratory findings negative, which subsequent observation demonstrated as nonluetic in character.

Thus in thirty-four cases of primary chancre in which the local Wassermann reaction was positive, only ten exhibited a positive blood reaction, one case being weakly positive and the remaining twenty-three cases showing a negative reaction in the blood serum. These findings agree with the results

of Klauder and Kolmer, who found that five cases out of twelve having both positive dark-fields and positive local Wassermanns, exhibited negative Wassermann reactions with the blood serum. As early as the third day after the appearance of the primary chancre, we found a positive reaction with the chancre serum. Kolmer and Klauder report a case of a seven day chancre with the local Wassermann reaction positive, in which the blood reaction was negative with three antigens. These cases strongly support the conception of a local, as well as hematogenous, origin of the specific complement-fixing antibody.

*Presence of Spirochetes.*—Strikingly constant is the occurrence of a positive local Wassermann reaction without exception in every case of demonstrated primary chancre. The local Wassermann parallels the dark-field findings, and is independent of the blood Wassermann reaction, the presence or absence of adenopathy or secondary manifestations, or the duration of the chancre itself. These findings suggest that the local complement-fixing body may occur only in the presence of *Spirochete pallida* in the lesions, but Klauder and Kolmer report five cases yielding positive local Wassermann reactions in which spirochetes were not demonstrable in the chancre. The parallelism between the presence of spirochetes and a positive local reaction can be best interpreted by concluding that the local Wassermann reaction is positive in a very high percentage, if not all cases, of true primary chancre.

*Effect of Local Treatment.*—The effect of local treatment is illustrated by case 17, which had been treated with iodoform, but still gave a positive reaction with the chancre fluid. In this case the spirochetes were still present, but it is usually difficult for spirochetes to be found from the lesion which has been treated locally. Klauder and Kolmer report a case in which the spirochetes were absent but in which the chancre fluid gave a positive reaction, although the lesion had been treated with a spirocheticidal drug. It is in this type of case that a positive local Wassermann reaction would be the only certain diagnosticating finding.

*Dilution of Serum.*—In amounts of 0.1 and 0.0125 c.c. the local Wassermanns were uniformly positive; with only 0.00625 c.c. there were twenty positives, six weak positives and ten negatives; with 0.004 c.c. there were only two positives, five weak positives and twenty negative reactions. Taking into consideration the varying dilution of the chancre serum with the saline with which the lesion is washed, the amount of serum fixing complement is comparable to the amount giving a positive reaction with blood serum, that is, approximately 0.001 c.c. Since positive reactions were obtained uniformly with 1 to 8 dilutions of the original 0.1 c.c., it seems reasonable to suppose that obtaining the serum by adding four or five drops of saline to the surface fluid and then pipetting off the mixture would give a strong enough concentration of antibodies to yield positive reactions.

#### SUMMARY

The Wassermann reaction carried out on the surface serum of a suspected primary lesion is a practicable and valuable aid in the early diagnosis



TABLE I

CASE	PRIMARY CHANCERE					WASSERMANN ON CHANCERE SERA					REMARKS	
	DAYS* DURA- TION	DAYS** EX- POSURE	LOCATION	ADENOP- ATHY	SEC- ONDARY LESIONS	BLOOD WASS.	DARK FIELD	0.1 c.c.	0.0125 c.c.	0.00625 c.c.		0.004 c.c.
1	T. O.	21	Corona	Inguinal	None	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Chancre treated with iodoform.
2	H. C.	21	Lip	Inguinal	None	Pos.	Pos.	Pos.	Pos.	Neg.	—	
3	W. S.	30	Corona	Inguinal	None	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.	
4	R. G.	—	Corona	Inguinal	None	Pos.	Pos.	Pos.	Pos.	Pos.	—	
5	H. L.	31	Corona	General	Papular	Pos.	Pos.	Pos.	Pos.	Pos.	—	
6	M. B.	46	Corona	Inguinal	Macular	Pos.	Pos.	Pos.	Pos.	Pos.	Weak pos.	
7	M. J.	18	Lip	General	Macular	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	
8	B. G.	20	Corona	Inguinal	Macular	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.	
9	J. S.	35	Corona	Inguinal	None	Pos.	Pos.	Pos.	Pos.	Pos.	Weak pos.	
10	M. T.	21	Corona	Inguinal	None	Pos.	Pos.	Pos.	Pos.	Pos.	Weak pos.	
11	G. G.	14	Lip	General	None	Pos. <sup>†</sup>	Pos.	Pos.	Pos.	Pos.	Weak pos.	
12	B. B.	21	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Pos.	Neg.	
13	J. S.	10	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Pos.	—	
14	W. F.	14	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Weak pos.	Neg.	
15	F. C.	24	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Pos.	Neg.	
16	G. J.	28	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Weak pos.	Neg.	
17	B. H.	10	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Pos.	—	
18	R. G.	—	Genitalia	General	None	Neg.	Pos.	Pos.	Pos.	Pos.	—	
19	E. J.	—	Corona	None	None	Neg.	Pos.	Pos.	Pos.	Pos.	—	
20	I. J.	10	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Pos.	Neg.	
21	V. J.	7	Lip	General	None	Neg.	Pos.	Pos.	Pos.	Weak pos.	Neg.	
22	M. D.	52	Lip	Cervical	Macular	Neg.	Pos.	Pos.	Pos.	Neg.	Neg.	
23	T. M.	14	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Pos.	Neg.	
24	H. L.	6	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Neg.	Neg.	
25	H. C.	8	Lip	Cervical	None	Neg.	Pos.	Pos.	Pos.	Pos.	Weak pos.	
26	S. A.	3	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Neg.	Neg.	
27	B. W.	10	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Neg.	Neg.	
28	M. C.	4	Lip	Cervical	None	Neg.	Pos.	Pos.	Pos.	Neg.	Neg.	
29	H. S.	—	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Neg.	Neg.	
30	B. C.	17	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Weak pos.	Neg.	
31	F. L.	4	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Weak pos.	Neg.	
32	H. V.	7	Genitalia	None	None	Neg.	Pos.	Pos.	Pos.	Weak pos.	Neg.	
33	J. Y.	4	Corona	Inguinal	None	Neg.	Pos.	Pos.	Pos.	Weak pos.	Neg.	
34	A. T.	5	Lip	None	None	Neg.	Pos.	Pos.	Pos.	Neg.	Neg.	
35	J. Y.	21	Genitalia	None	None	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	
36	G. H.	10	Genitalia	None	None	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	

\*Days from first appearance of chancre.

\*\*Days between first appearance of chancre and exposure.



TABLE II  
SUMMARY OF RESULTS

NO. CASES	LESION	BLOOD WASSERMANN	SECOND- ARIES		DARK FIELD	WASSERMANN ON CHANCERE SERA											
						0.1 cc.		0.0125 cc.		0.00625 cc.		0.004 cc.					
			Pres.	Abs.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Weak	Neg.	Pos.	Weak
10	Primary chancre	Positive	4	6	10	0	10	0	8	0	2	2	2	3			
1	Primary chancre	Weak pos.	-	1	1	-	1	-	1	-	-	-	-	1			
23	Primary chancre	Negative	1	22	23	0	23	0	11	6	6	0	0	2	18		
2	Chancroids	Negative	0	2	0	2	0	2	0	0	2	0	0	0	1		
36			5	31	34	2	34	2	20	6	10	2	5	23			

of primary syphilis. We do not in any sense suggest this reaction as a substitute for the dark-field, which should be employed in all cases. But in cases in which no dark-field microscope is available, in cases in which the dark-field examination is negative, and in cases, particularly, where treatment of a lesion with a spirocheticide has driven the spirochetes from the chancre, the local Wassermann reaction should be employed. In such circumstances the local Wassermann reaction is the only positive diagnostic procedure available earlier than the appearance of a positive blood reaction, which does not occur until there is systemic invasion. Because of the very great importance of a prompt diagnosis, this simple procedure has a definite place in the diagnosis of primary syphilis in the earlier stages.

The local Wassermann was positive in all of our thirty-four cases of demonstrated primary chancre, in the majority of which the blood Wassermann was negative and secondary manifestations were absent. A local as well as hematogenous origin of the specific complement-fixing antibodies appears definite. In all cases 0.0125 c.c. of the serum was sufficient to give the positive reaction. The simplicity with which such a small amount can be obtained from the surface of a chancre by diluting with several drops of saline, disposes of any suggestion of difficulty in securing sufficient serum to carry out the reaction. In a later communication we hope to report on the practicability of preserving and mailing tubes of serum to distant laboratories for the performance of the Wassermann test.

#### CONCLUSIONS

1. The Wassermann reaction carried on the chancre sera of 34 demonstrated cases of primary chancre was positive in all cases.
2. Of the 34 cases giving positive local Wassermann reactions the majority showed negative blood reactions and no secondary manifestations.
3. The local Wassermann reaction was positive with 0.0125 c.c. in all cases and in some cases with only 0.004 c.c. of chancre serum.
4. Treatment of the lesions locally with a spirocheticide appears to have no effect upon the complement-fixing antibodies, although the spirochetes may disappear.
5. A local as well as hematogenous site of origin for the specific complement-fixing antibodies appears established.
6. In cases where no dark-field microscope is available, where the spirochetes are not found, or where a lesion has been treated by a spirocheticide, the local Wassermann reaction is a practicable, simple and valuable means of diagnosis of primary syphilis at the time most important for prognosis and treatment.

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# THE ACTION OF PROTEINS AND BLOOD SERUM ON COLLOIDAL GOLD SOLUTION AND ITS QUANTITATIVE INTERPRETATION\*

BY PAUL REZNIKOFF, M.D., NEW YORK CITY

THE INTERPRETATION of the colloidal gold reaction has occupied the attention of investigators since its introduction. Although Lange believed the phenomenon to be one of protein chemistry, the effect of the individual proteins in cerebrospinal fluid upon the colloid, other views were entertained and experimental evidence was presented to support them. Scant attention need be paid to the inorganic elements of cerebrospinal fluid or blood serum. The concentration of inorganic electrolytes is negligible with respect to any action on colloidal gold solution. The consideration of ferments or specific antibodies as the factors in the production of the curves can have but little weight in view of the very definite and conclusive action of the proteins upon colloidal gold.

Zsigmondy introduced his work on colloidal solutions with the very significant statement that although Faraday preceded him by many years in his experiments on colloids, this was rather an incentive than a deterrent. He pointed out that no research can be found which has not been touched upon by some former worker usually unknown to the new enthusiast. In this very field the problems which are still important and unsolved have been studied by the pioneers in colloidal chemistry. Lange, himself, used blood serum on colloidal gold solution and drew conclusions from his meagre results.

The exact action of the proteins upon colloidal solutions is more than of academic interest. Our methods for the isolation and study of these protein bodies are very unsatisfactory. The best determinations are essentially approximate. That the proteins play a very important part in our metabolic processes can be of very little doubt. Even rough estimates of the total and individual blood serum proteins point to marked differences in certain clinical conditions.

## PURE PROTEINS

With these considerations in view pure proteins† were tested against colloidal gold and definite curves obtained. These proteins,—serum albumin,

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†The euglobulin was prepared by precipitation from fresh serum with a saturated solution of sodium chloride. The pseudoglobulin was prepared by precipitating both the euglobulin and the pseudoglobulin by half saturation with ammonium sulphate and removal of the pseudoglobulin by washing with saturated sodium chloride. These steps were repeated several times. The resulting proteins contained the faintest trace of the other globulin according to a precipitin test, but they were found to be the purest globulins that could be obtained by separation with salts. Of course, we have no evidence that the globulins are distinct immunological entities and cannot rule out chemical purity by the precipitin test. The albumin was found to be free from other proteins.

serum pseudoglobulin and serum euglobulin, were obtained from Dr. Arthur F. Coca, of the Department of Immunology of the New York Hospital. Preliminary work was done with proteins secured from the Bureau of Laboratories, Department of Health, New York City, and certain commercial products. The pure albumin contained 3.36 mg. nitrogen per c.c., was in aqueous solution and could be tested directly against the colloid. The pseudoglobulin and euglobulin were not dialysed to be freed from the excess salt but were precipitated with 95 per cent alcohol, centrifuged, washed with 80 per cent alcohol three times, centrifuged each time, and then washed into a dish with 95 per cent alcohol. The mixture was dried by an electric fan and the globulins taken up with 0.4 per cent saline. The resulting solutions contained 0.448 mg. pseudoglobulin nitrogen per c.c. and 0.35 mg. euglobulin nitrogen per c.c. The colloidal gold solution was prepared according to the method of Lee. It was found that a constant solution, neutral to alizarin, was obtained which was very sensitive, normal cerebrospinal fluids giving a curve. The reason for this extreme sensitivity was probably the excess and complete addition of formaldehyde before heating; but this was found to be desirable because of the constancy of the various lots of solution. The details of cleaning the glassware were adhered to as carefully as for the regular colloidal gold test.

The albumin-colloidal gold reaction was tested by setting up twenty-nine tubes. In the first, 2 c.c. of the stock solution was placed, in the second, 1 c.c., in the third, 1 c.c., and in each of the remaining tubes 0.5 c.c. of 0.4 per cent saline. 0.5 c.c. of the albumin solution was removed from the third tube and put into the fourth. The saline and albumin were thoroughly mixed and 0.5 c.c. of the mixture was put into the fifth tube. This procedure was repeated throughout all the tubes until the last, where the 0.5 c.c. of the mixture was discarded. The resulting series, therefore, started with an albumin nitrogen content of 6.72 mg. in the first tube, 3.36 mg. in the second, 1.68 mg. in the third, and so on. By diluting each succeeding tube with an equal quantity of saline as indicated above, the twenty-ninth tube contained 0.00000002 mg. of albumin nitrogen. Each tube thus contained half the quantity of albumin of the preceding tube. Only twenty-nine tubes were used because preliminary tests showed that after that no further reaction was obtained. Five c.c. of colloidal gold solution was added to each tube and the tube was thoroughly shaken. The first tube to decolorize completely was noted, marked "x" on the charts, and the reaction was read in twenty-four hours. In the reading, the same numbers were used to refer to the colors as in the regular Lange reaction, namely:

Red	(0)	Blue	(3)
Red-blue	(1)	Light blue	(4)
Violet	(2)	Colorless	(5)

The greater the precipitation of the colloidal gold, the higher the number reads. (5) represents complete precipitation.

The pseudoglobulin test involved the use of twenty-four tubes. Here, 3 c.c. of the pseudoglobulin solution was used in the first tube, giving 1.344



mg. nitrogen of this protein, 2 c.c. in the second, 1 c.c. in the third and fourth, and so on, as outlined in the albumin test. The dilutions were brought up to 0.5 c.c. with 0.4 per cent saline except for the first three tubes which contained 3 c.c., 2 c.c. and 1 c.c. respectively of the original 0.4 per cent saline solution of the pseudoglobulin. Here again, 5 c.c. of colloidal gold solution was added and readings made as for the albumin test.

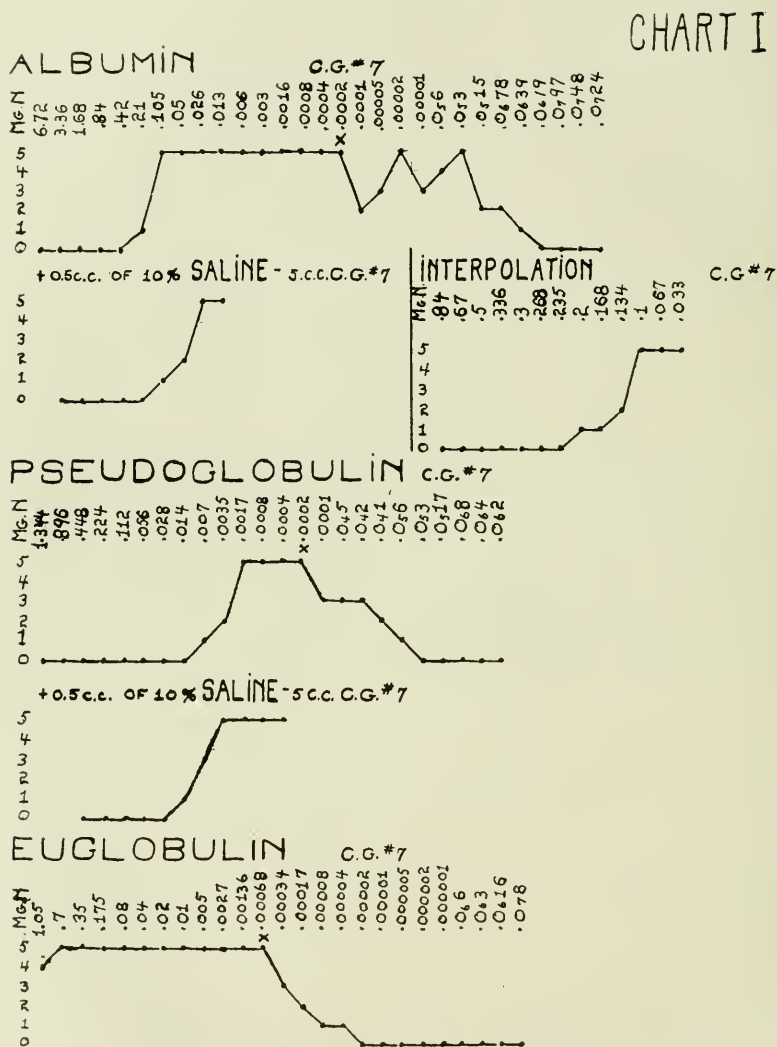


Chart I.

The euglobulin tubes numbered twenty-five. The first tube contained 3 c.c. of the euglobulin solution, containing 1.05 mg. nitrogen of this protein; the second containing 2 c.c., the third, 1 c.c., and so on, as indicated above, the dilutions of all the tubes except the first three being 0.5 c.c. Here again, the readings were made as for the two preceding proteins.

For albumin an interpolation test was performed through the phase

where the first decolorization appears. Instead of halving the amount of albumin in each succeeding tube, definite amounts were made up thus: 0.25 c.c., 0.2 c.c., 0.15 c.c., 0.1 c.c., 0.09 c.c., 0.08 c.c., 0.07 c.c., 0.06 c.c., 0.05 c.c., 0.04 c.c., 0.03 c.c., 0.02 c.c., 0.01 c.c. and the result was noted.

The results obtained are given in the accompanying Chart I. The curves are seen to be distinctive for each type of protein. In the curves the abscissae represent the extent of decolorization; the dots on the ordinates, the tubes. "C.G.#" indicates the lot of colloidal gold solution used and the number of milligrams of nitrogen of the protein for each point on the curve is represented above the curve.

The curve obtained for serum albumin shows the following characteristics: From 6.72 to 0.21 mg. no precipitation occurs; at 0.21 mg., the precipitation begins (1). 0.1 mg. of albumin nitrogen precipitates the 5 c.c. of colloidal solution completely, a colorless tube resulting. At 0.0002 mg. the decolorization ceases and the curve tends to zero. But two secondary rises develop, at 0.00002 mg. and 0.000003 mg. the curve going up to (5). At 0.00000019 mg. the color is again red and remains so throughout further dilutions. The tube to decolorize completely most rapidly is the sixteenth containing 0.0002 mg. of serum albumin nitrogen.

The serum pseudoglobulin curve shows no precipitation until 0.007 mg. nitrogen of the protein is reached. Decolorization occurs at 0.0017 mg. and after 0.0002 mg. precipitation diminishes so that at 0.000003 mg. the curve reaches the base line and remains flat on further dilution. The first tube to decolorize completely for this protein is 0.0002 mg. nitrogen.

Serum euglobulin does not protect the colloidal solution in the highest concentrations obtainable. The curve starts at (4) and at 0.7 mg. nitrogen precipitates the colloid completely. After 0.00068 mg. the curve sinks and at 0.00002 mg. reaches a red color, never rising upon further dilution. The first tube to decolorize completely in point of time is the one containing 0.00068 mg. of euglobulin nitrogen.

Besides the definite quantitative action upon colloidal gold of known amounts of the proteins, the curves of each have their own individual characteristics. Serum albumin begins to precipitate colloidal gold solution in relatively high concentration. The phase of transition from the beginning to complete precipitation is a short one, whereas the phase of complete precipitation is long. Finally there are two secondary rises which are constant.

Pseudoglobulin does not begin to precipitate colloidal gold until in rather dilute solution. The rise of the curve is rapid and the phase of complete precipitation is very short. The descent is gradual and there are no secondary rises.

Euglobulin is characterized by the absence of a protective phase in even the most concentrated solutions that can be obtained for use against colloidal gold solution. The phase of complete precipitation is a long one, but having reached zero, the curve remains flat.

All the proteins have one feature in common, namely, the tube with the least amount of protein to decolorize completely is the first one to decolorize

with respect to time. Then the decolorization continues to the left. This is seen most commonly in a paretic cerebrospinal fluid curve.

The protective mechanism of the greater concentrations of albumin and pseudoglobulin is a well-known colloidal phenomenon. It is seen in the prozone of a precipitin reaction. The secondary rises of albumin, or perhaps more correctly, the two descents before the final permanent descent of the curve is probably a type of prozone.

*Gold Number.*—In connection with this work it is of interest to consider the so-called gold number. Zsigmondy defined this as the number of milligrams of a colloid that protects 10 c.c. of colloidal gold solution from further change after 1 c.c. of a 10 per cent sodium chloride solution is added. In the case of these proteins 5 c.c. of colloidal gold and 0.5 c.c. of 10 per cent saline were used. The chart shows that for serum albumin the gold number lies somewhere between 0.2 and 0.026 mg. nitrogen for the particular colloidal gold solution used. For pseudoglobulin the gold number lies between 0.02 and 0.0035 mg. nitrogen. Euglobulin has no gold number, failing to protect the colloidal gold from the 10 per cent saline in any concentration. This could be readily surmised from the character of the curve. It is noteworthy that albumin and 10 per cent saline have a higher protective action on colloidal gold than when albumin is used alone. This is an illustration of the fact that the combined action of electrolytes may not be cumulative but entirely opposite in effect from their action when used individually.

*Protein Mixtures.*—Combinations of the proteins were next tested. The first mixture consisted of 1 mg. albumin nitrogen, 0.5 mg. of pseudoglobulin nitrogen and 0.5 mg. of euglobulin nitrogen in the first tube, half these amounts in the second and so on. The resulting curve on Chart II shows a marked resemblance to euglobulin in the failure of the high concentrations to protect and the position of the phase of decolorization in the curve. Furthermore, the total number of milligrams of nitrogen in tube twelve, the first to decolorize in point of time, is 0.0009, resembling euglobulin in this respect. The effect of albumin is seen in the slight drop of the thirteenth tube to (4).

A second combination was made from the stock aqueous and 0.4 per cent saline solutions containing 2.25 mg. albumin nitrogen, 2.5 mg. pseudoglobulin nitrogen and 1.75 mg. euglobulin nitrogen. This was in accordance with a verbal report of the relative amounts of these proteins in normal horse serum found in the Laboratories of the Board of Health. A mixture of 0.67 c.c. of the albumin, 5.58 c.c. of the pseudoglobulin and 5 c.c. of the euglobulin solutions gave the above results. Using half the above mixture in the first tube, one quarter, in the second and halving the amounts in succeeding tubes, the number of milligrams of nitrogen in the first tube was 1.125 albumin, 1.25 pseudoglobulin and 0.875 euglobulin.

The curve, shown on Chart III presents four features. (a) There is a failure to completely protect in the greatest concentration used. The curve starts at (1) instead of (0) showing the euglobulin effect. (b) There is a long continued complete precipitation phase, resembling in extent and posi-

tion, the albumin curve. (c) The first tube in time and lowest in concentration to completely precipitate contains a total of 0.00019 mg. of nitrogen, the amount giving the same result in the albumin and pseudoglobulin curves. (d) The albumin effect is seen still further in the secondary rise. It can be seen that this mixture is dominated by albumin. Pseudoglobulin, apparently, has little effect because of the very nature of its curve. If anything, it has

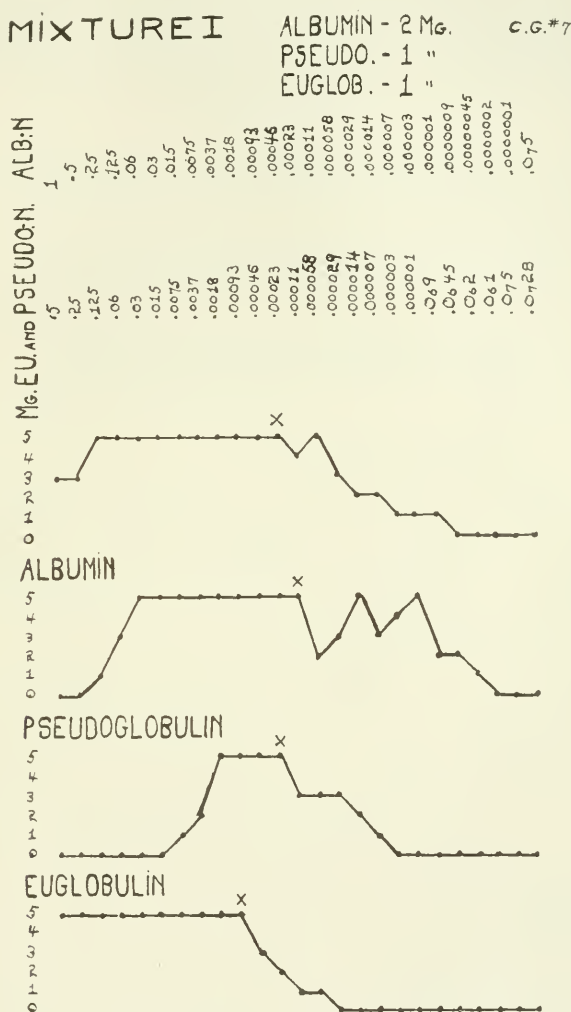


Chart II.

a protective action in the higher and lower concentrations, tending to overcome the effect of euglobulin in the higher concentrations and that of the secondary rises of albumin in the dilute mixtures.

*Blood Serum.*—Before undertaking the study of the effect of blood serum on colloidal gold solution, deproteinized serum was tested. Lange thought that there were no differences between the various blood sera curves, but when these sera were deproteinized he thought that luetic sera always showed much





4. Tungstic acid precipitates colloidal gold completely.
5. Trichloroacetic acid precipitates colloidal gold completely.
6. Colloidal iron - filtrate is colored.

The only agent that precipitated protein completely, so that the filtrate when evaporated to dryness gave no Biuret or Millon's test, and which itself neither precipitated nor protected the gold solution was alcohol. Ninety-five per cent alcohol was used in the proportion of 9 c.c. to 1 of serum and after thorough mixing was permitted to stand for twenty-four hours and filtered. The serum used in all cases was free from the slightest visible hemolysis.

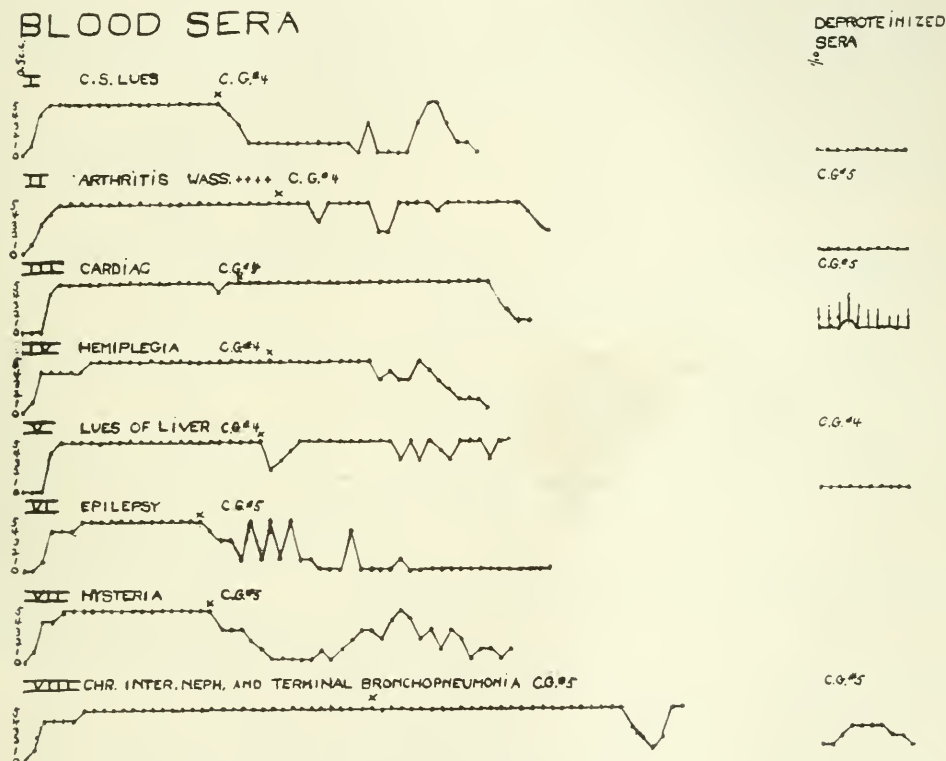


Chart IV.

On the right hand side of Chart IV, V, VI the results of deproteinized sera and colloidal gold are given. One c.c. of the 1-10 alcoholic filtrate was placed in the first two tubes. Five-tenths c.c. of 0.4 per cent saline was added to eight remaining tubes. Five-tenths c.c. of the alcoholic filtrate was removed from the second tube to the third and this process was continued through the remaining tubes so that the resulting tests represented 1/10 c.c. of the original serum in the first tube, 1/20 c.c., in the second and so on, the tenth tube being equivalent to 1/5120 c.c. of the serum which had been deproteinized. It can be seen that in one case the curve goes up to 1 and in two cases to 2 (Cases III, VIII, XI). In all the other cases the curves are flat indicating no protein. The slight rises in the curves men-

tioned indicate only the faintest traces of protein when the extreme sensitivity of the colloidal gold solution used is considered. Furthermore, in these three cases, as will be pointed out later, the curves of the original serum showed a marked increase in protein content. A rise of 2 with ordinary colloidal gold solution is considered high normal in testing cerebrospinal fluid. It must be remembered that the colloidal gold used in these experiments was of such delicacy that it gave a positive curve as high as 5 with normal cerebrospinal fluid. It may then be considered that serum deproteinized by alcohol has no action on colloidal gold solution except in those sera with marked increase of protein where apparently the slightest traces remain.

The sera from eight patients were taken, all at the same time of day. These cases were chosen at random to see the effects of the serum on colloidal gold solution. The tests were performed as for the pure proteins. One c.c. of the serum was placed in the first tube, 0.5 c.c. of 0.4 per cent saline in each

## BLOOD SERA

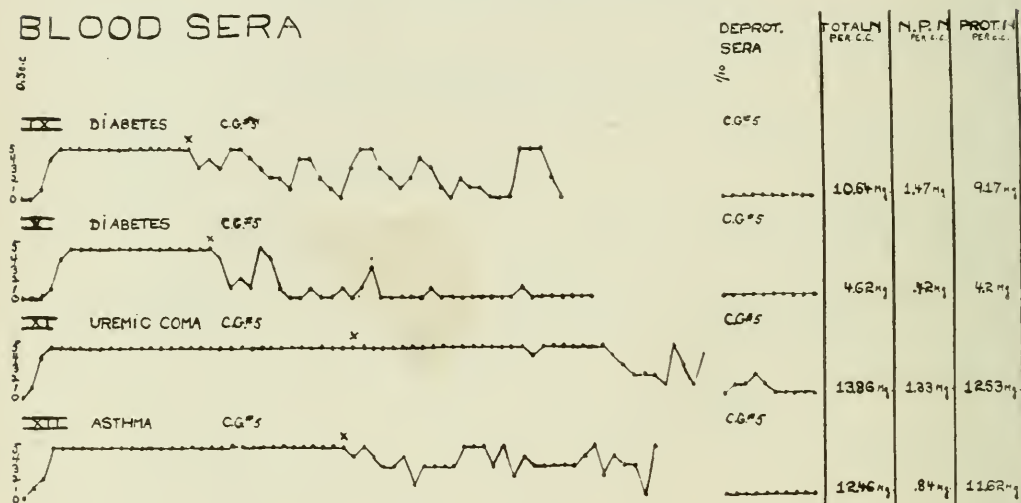


Chart V.

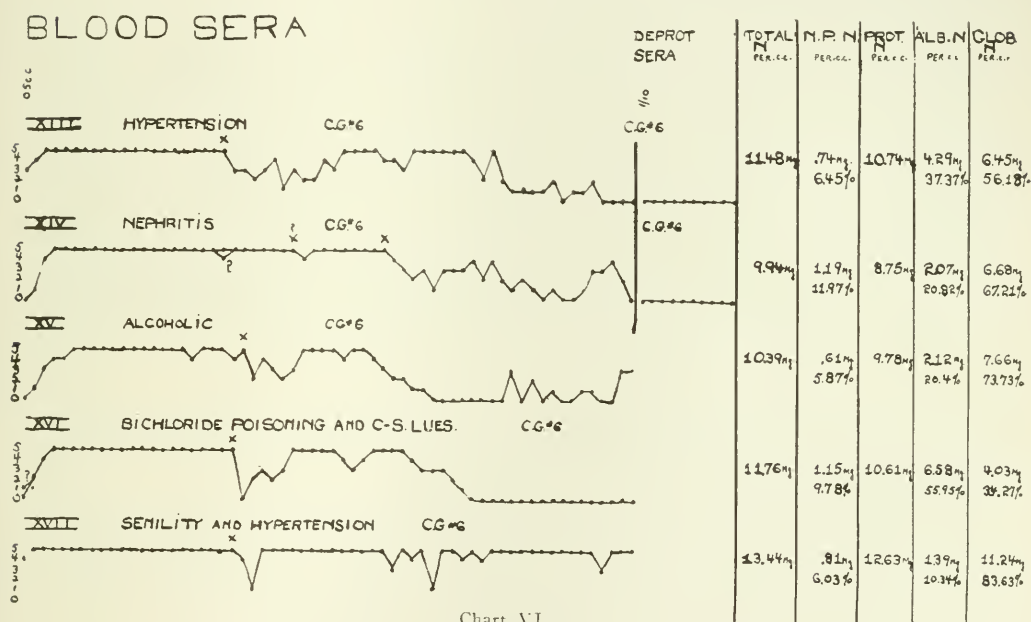
of the remaining tubes, as a rule sixty-one tubes being used. Five-tenths c.c. of the serum was transferred from the first tube to the second and so on: so that 0.5 c.c. of the original serum was contained in the first tube, 0.25 c.c., in the second, etc. All the dilutions were made up to 0.5 c.c. with 0.4 per cent saline. Five c.c. of the colloidal solution was added to each tube. Chart IV shows the marked variation of curves obtained. Practically no two curves resembled each other except for general appearance,—such as Cases I and VII. Case VIII was one of terminal chronic interstitial nephritis and bronchopneumonia and represents an extreme type of curve.

The factors involved in these results are: (a) the total amount of protein, (b) the amounts of individual proteins, (c) the combinations of individual proteins in each dilution. Therefore, a simple analysis and explanation of a curve is hardly possible because of the very nature of its complexity.

To obtain some idea of at least one of these factors, four cases were taken to determine the serum curve, the total nitrogen, the nonprotein nitrogen

in the alcoholic filtrate and thus the total protein nitrogen. Chart V shows the relationship of the curve to the total protein nitrogen. The protein nitrogen varied from 4.2 mg. per c.c. to 12.53 mg. per c.c. The higher the protein content, the more sustained the curve, that is, the more tubes completely decolorized. Curve XI is a case of uremic coma and shows the most marked effect of the protein. Curve X, a diabetic of long standing shows the least effect. It can readily be seen that the nonprotein nitrogen has no effect, case IX having a serum nonprotein nitrogen of 1.47 mg. per c.c. and case XI of 1.33 mg. The total protein content had, therefore, a marked effect on the "intensity" of the curve.

The other factors involved in the production of a colloidal gold curve with serum are not easily determined. There is no chemical method for determining proteins which is accurate and practical. Howe's sodium sul-



phate method was tested against the pure proteins to determine its applicability to these experiments. It was found that neither 14 per cent nor 22.2 per cent sodium sulphate had any effect on the albumin; that the 14 per cent salt precipitated 97.88 per cent of the euglobulin; that the 22.2 per cent salt precipitated 96.04 per cent pseudoglobulin; but that the 14 per cent solution precipitated 89.16 per cent of the pseudoglobulin, thus making the determination of the separate globulins impossible.

An analysis of the curves shown on Chart VI cannot be made in great detail. In general, the intensity of curve depends on the total protein content, —curve XVII showing this most markedly with 12.63 mg. of protein nitrogen per c.c. of serum. The proportion of albumin in the sera was found to be lower than is usually given, varying from 10.34 per cent to 55.95 per cent of the total nitrogen. Curve XIII in all probability indicates a large amount



of euglobulin, by the failure of the curve to start at 0, but beginning at 3. Curve XVI shows best two distinct secondary rises and would thus indicate relatively more albumin. This is actually the case, 55.95 per cent of the total nitrogen belonging to that protein. Curve XVII shows 83.63 per cent globulin nitrogen, and indicates much euglobulin, the start taking place at 4.

No real explanation of the effect of serum on colloidal gold can be made, however, until simple and accurate chemical methods for separating and determining the proteins are found. The importance of obtaining such methods can be seen in the great variation of total and individual protein content of the sera in the different clinical conditions indicated on the charts. Probably more proteins play a part in the production of the curve than we can now isolate and determine. The great variation in total protein content of blood sera is in itself a subject that has been given little attention and may be of considerable importance.

While these tests were in progress, Heinrich Fischer published an account of his elaborate and detailed experiments on the mechanism of the colloidal gold reaction in cerebrospinal fluid. His methods involved separation of proteins by means of ammonium sulphate and preparation of his colloidal gold with the aid of glucose. His results show that the curves obtained were due to the action of proteins. In one finding there is wide disagreement from the results obtained in this work. He finds albumin has no effect on colloidal gold, but actually protects the solution from other proteins and electrolytes. In the experiments described in this paper albumin was found to have a very distinct and characteristic curve.

#### SUMMARY AND CONCLUSIONS

1. Solutions of pure serum albumin, serum euglobulin and serum pseudoglobulin were tested against colloidal gold solution and were found to have definite and characteristic curves for each protein.

2. These curves depend upon the concentration of the protein in solution that acts as a protecting or precipitating agent of the colloidal gold.

3. A gold number was obtained for the serum albumin and serum pseudoglobulin, with the colloidal gold solution used in these tests.

4. Mixtures of the three proteins were tested with the colloidal gold solution and an analysis of the curves obtained showed the influence of the individual proteins depending on their concentrations in the mixtures and upon their normal action.

5. (a) Blood serum can be deproteinized with 95 per cent alcohol and the filtrate obtained will have no effect on colloidal gold or in extreme cases a negligible effect.

- (b) The colloidal gold reaction is a protein phenomenon.

6. Human sera show marked differences in their effect on colloidal gold solution in various clinical conditions.

7. The total protein content of a serum is an important factor in maintaining a curve. The "intensity" of the curve varies directly with the amount of protein present.

8. In various clinical conditions the amount of protein nitrogen and the percentages of albumin and globulin found in sera vary greatly.

9. The curves are influenced by the variations in albumin-globulin ratio.

10. Howe's method for isolating the globulins was not found to be accurate by testing it with the pure proteins used in these experiments, the euglobulin precipitating agent precipitating 89.16 per cent of the pseudoglobulin.

11. The results obtained with serum albumin show it to have a distinct precipitating effect on colloidal gold in certain definite concentrations, thus differing from the findings of Fischer.

12. New methods for the separation and determination of proteins are necessary before the very obvious and probably important differences of these substances in various clinical conditions can be investigated.

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# THE CENTRAL INFLUENCE OF ATROPINE AND HYOSCINE ON THE HEART RATE\*

BY W. J. R. HEINEKAMP, CHICAGO, ILL.

RECENTLY McGuigan<sup>1</sup> reported that pharmacopoeial doses of atropine, 0.0005 gm., cause slowing of the heart only, when given to adults. This he believed to be the result of central stimulation. Large doses, however, paralyze the vagus endings almost immediately so that the central action of atropine is unable to influence the heart. Harris<sup>2</sup> confirmed McGuigan's findings and states that doses of 0.00065 gm. in man were not strong enough to effect paralysis of the vagus endings, but that the slowing which occurred was evidently due to vagus stimulation. Paskind,<sup>3</sup> in a series of experiments, found that while a small dose of atropine causes slowing of the heart in white adults, as a rule it produces acceleration in negroes.

Sollmann<sup>4</sup> states that atropine stimulates the vagus center in common with other medullary centers and hence may cause a primary slowing. Cushny<sup>5</sup> states that "even therapeutic doses slow the pulse for a short time apparently from direct action on the heart." Also, that the action of atropine on the vagus endings is purely depressant (*loc. cit.* p. 12). Bush<sup>6</sup> using 0.02 per cent atropine found that it exerted little or no influence on the cardio-inhibitory center of the turtle.

Hyoscine, according to Cushny<sup>7</sup> does not alter the pulse, except that it may be slower, owing to the hypnotic action. Sollmann states that the pulse may be unchanged, or considerably slowed from central vagus stimulation.<sup>8</sup> I have found that atropine in small doses—0.0003 gm. when injected subcutaneously in the dog produces slowing of the heart. This action is not manifested after section of the vagi or with anesthesia, and hence is central. I have also found that atropine and hyoscine produce inhibition of the heart when perfused through the medulla of the yellow bellied terrapin (*Pseudomys troostii*). Hyoscine, however, also depresses the cardio-inhibitory center.

## EXPERIMENTS

### SERIES A

The methods used in perfusing the medulla were those reported in a previous article.<sup>9</sup> Atropine sulphate (Merek) and hyoscine hydrobromide (Merek) were used, dilutions being made up from 1 per cent solution.

The following experiments illustrate the various results obtained; namely:

1. Inhibition produced by atropine.
2. Inhibition produced by hyoscine.
3. Inactivity of strychnine and atropine after hyoscine.

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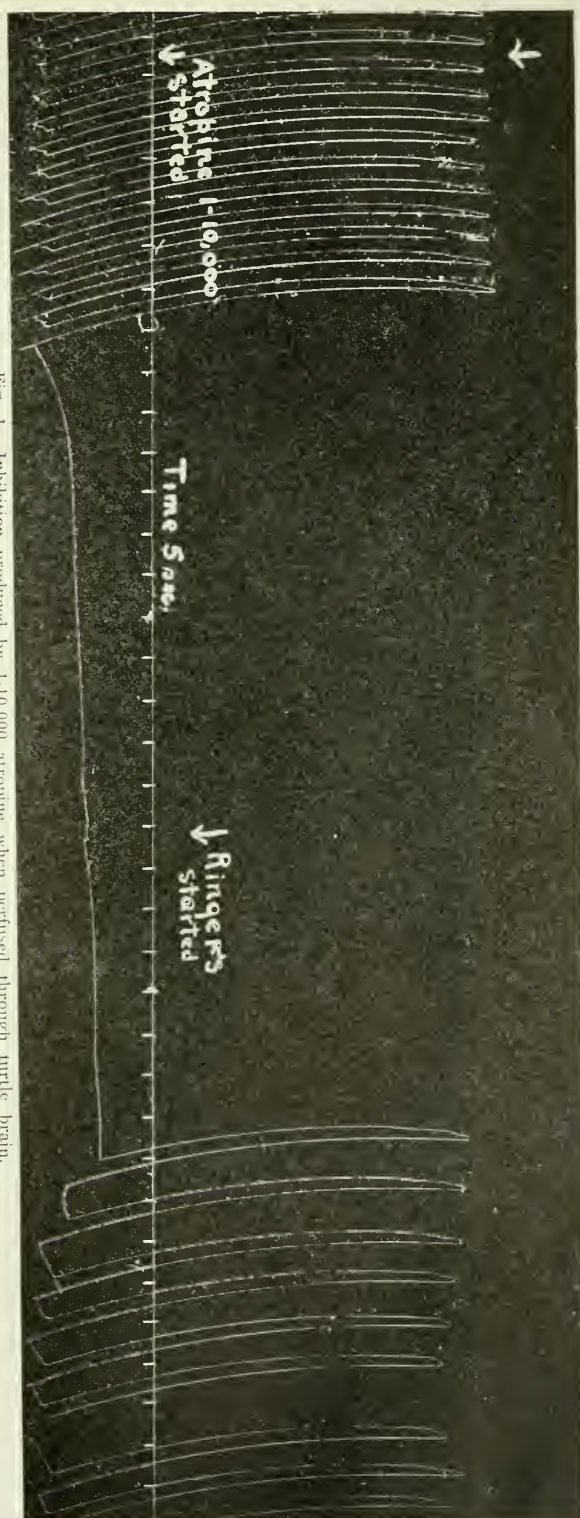


Fig. 1.—Inhibition produced by 1-10,000 atropine when perfused through turtle brain.

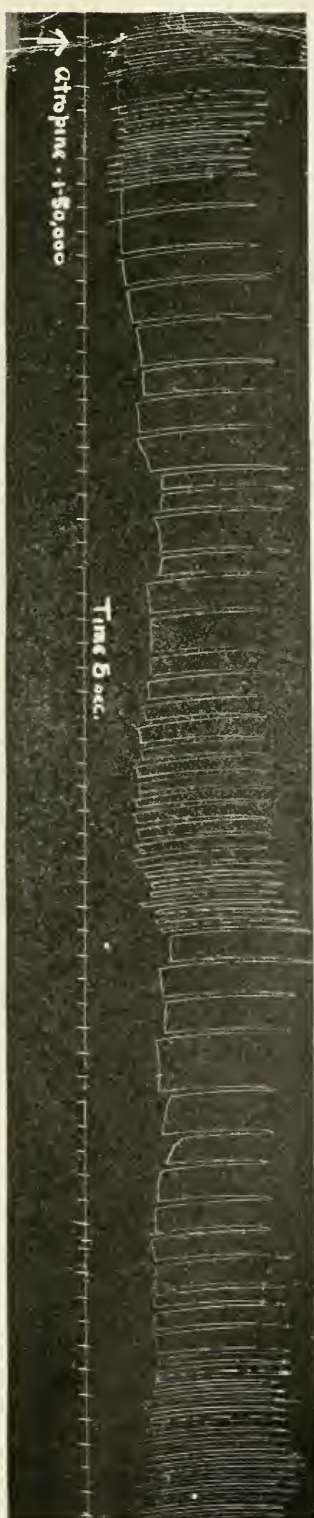


Fig. 2.—Inhibition produced by 1-50,000 atropine.





Fig. 3.—Inhibition produced by 1-100,000 atropine.

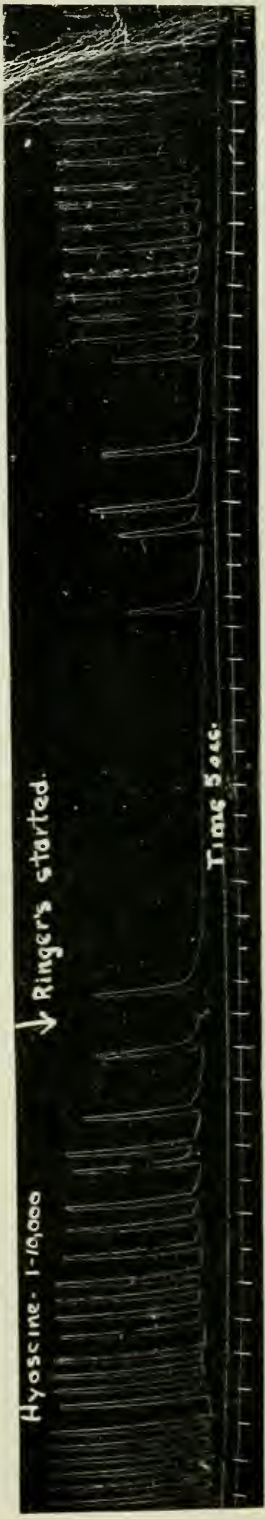


Fig. 4.—Inhibition produced by 1-10,000 hyoscine.

## EXPERIMENT 1.

Time (min.)	Remarks	Rate
-	Normal	34
9 2/3	After perfusion with 0.02 per cent atropine sulphate.	6
-	After perfusion with Ringer's	38
1/4	After perfusion with 0.01 per cent atropine sulphate.	8
-	After perfusion with Ringer's	36
1/2	After perfusion with 0.002 per cent atropine sulphate. Slowing persisted 12 minutes.	8
-	After perfusion with Ringer's	39
3	After perfusion with 0.001 per cent atropine sulphate.	10
-	After perfusion with Ringer's	37

## EXPERIMENT 2.

Time (min.)	Remarks	Rate
-	Normal	20
1/2	After perfusion with 0.01 per cent atropine sulphate.	0
-	After perfusion with Ringer's	26
2/3	After perfusion with 0.01 per cent atropine sulphate.	0
-	After perfusion with Ringer's	25
4	After perfusion with 0.002 per cent atropine sulphate.	24
1	After perfusion with 0.01 per cent atropine sulphate.	0
-	After perfusion with Ringer's	25
1	After perfusion with 0.01 per cent atropine sulphate.	0
-	After perfusion with Ringer's	28
2 1/2	After perfusion with 0.01 per cent hyoscyne hydrobromide. Slowing persisted 11 minutes	0
-	After perfusion with Ringer's	22
5	After perfusion with 0.01 per cent atropine sulphate.	24

## EXPERIMENT 3.

Time (min.)	Remarks	Rate
-	Normal	48
*2	After perfusion with 0.01 per cent hyoscyne hydrobromide	8
-	After perfusion with Ringer's	42
2	After perfusion with 0.01 per cent strychnine sulphate	10
-	After perfusion with Ringer's	50
-	After perfusion with 0.01 per cent hyoscyne hydrobromide	Irreg.
10	After perfusion with 0.01 per cent strychnine sulphate	46
14	After perfusion with 0.01 per cent atropine sulphate.	44

\*Heart had started to return to normal before Ringer's was used.

## SERIES B

In this series well-nourished dogs were injected subcutaneously with atropine sulphate in olive oil. The slow absorption kept the concentration such that while the cardio-inhibitory center was stimulated, the vagus endings were not paralyzed. When atropine was injected in a water solution, absorption occurred so rapidly that the slowing phase was either materially

shortened or did not occur at all. Inhibition did not occur in any experiment after section of the vagi, or while the animal was under the influence of ether anesthesia. It was noted that after the slowing phase the heart became very irritable and irregular, due to the mixture of central stimulation and peripheral depression. This was more marked when a water solution of atropine was used than when olive oil was used as a menstruum. Fig. 5 shows two typical experiments.

It is very necessary to keep the animal quiet throughout the entire ex-

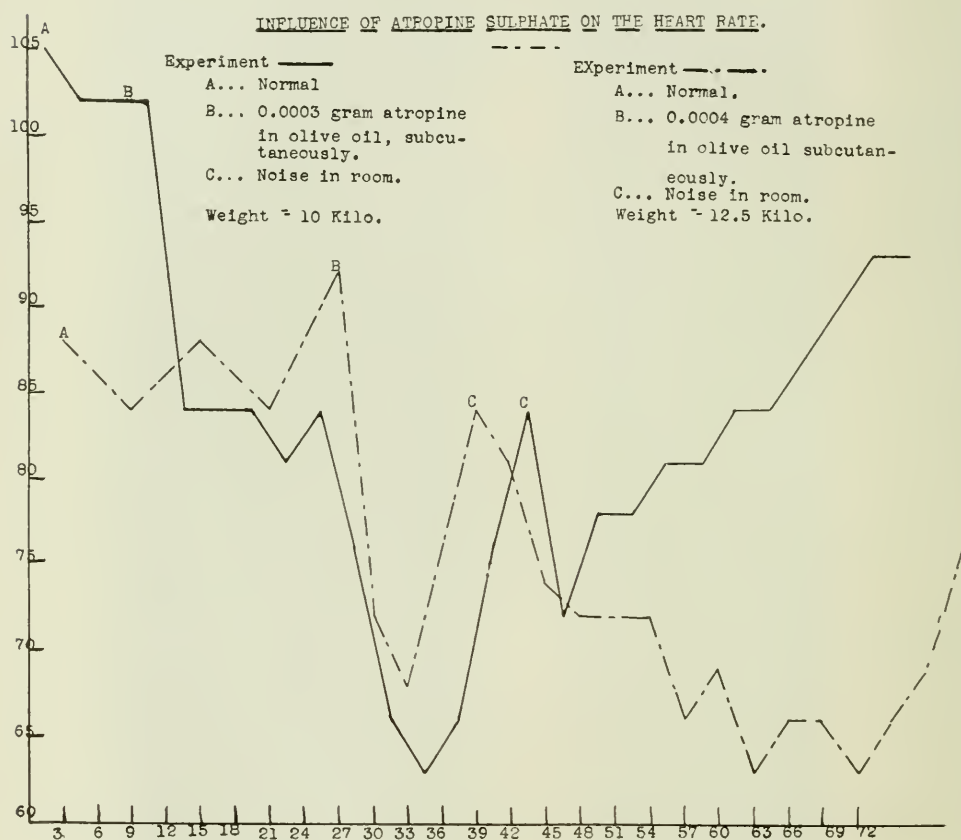


Fig. 5.—Chart showing influence of atropine sulphate on the heart rate of dogs.

periment since even slight noise causes a rapid rise in pulse rate, especially when atropine begins to depress the vagus terminations.

Fig. 6 is from the work of Dr. McGuigan. The patient was a well adult man, and was given 0.0005 gm. of atropine sulphate by mouth. Slow absorption probably accounts for gradual prolonged slowing.

Fig. 7 is from the work of Dr. Paskind, of this laboratory, and illustrates the difference in susceptibility to atropine which the white and negro race exhibit. Both subjects received 0.001 gm. of atropine sulphate subcutaneously. Negroes are relatively unsuceptible to the central action of atropine.

## DISCUSSION

Analysis of the results obtained in the above experiments shows that atropine sulphate in small doses causes inhibition of the heart. While this may be due to:

1. Stimulation of the cardio-inhibitory center.
2. Stimulation of the intrinsic ganglia of the heart.
3. Stimulation of either the parasympathetic or sympathetic nerve endings to the heart.
4. Direct action on the heart muscle; I believe that it is due to direct stimulation of the medulla.

Atropine exerts no influence on ganglion cells, except in massive doses when they are depressed along with all cells. After the injection of an amount of atropine sufficient to produce mydriasis, stimulation of the cervi-

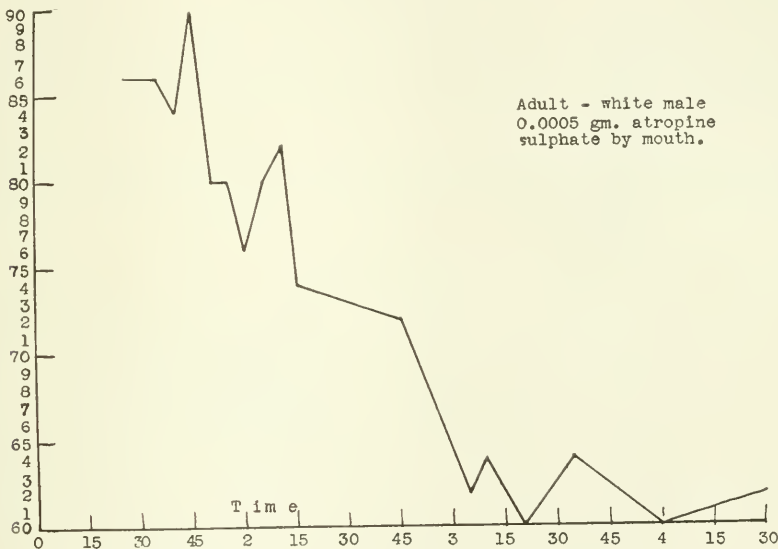


Fig. 6.—Chart showing the influence of atropine sulphate on the heart rate of an adult white and of an adult negro when given subcutaneously.

cal sympathetic nerve below the superior cervical ganglion still produces a further dilation of the iris, also constriction of the ear vessels, and a flow of saliva. These facts indicate clearly that atropine has no influence on ganglion cells, and hence the probability of atropine producing inhibition by stimulating the intrinsic ganglion of the heart, is excluded.

The influence of atropine directly on heart muscle is negligible. Gruber and Markel<sup>11</sup> state that atropine increases the general tonus of the terrapin heart muscle, but that it exerts only a slight effect on the tonus waves, increasing them if anything. That the slowing after the injection of atropine is not due to its action on the myocardium is proved by lack of inhibition after section of the vagi.

The peripheral action of atropine is characterized by depression of the parasympathetic myo-neural junctions. It exerts no influence on the sym-



pathetic endings to the heart. In my work on dogs I have found that after section of the vagus, or when an animal is under anesthesia, atropine will not produce inhibition.

The inhibition effected by atropine is due to stimulation of the cardio-inhibitory center. This is supported by the following facts. When atropine is perfused through the medulla of the yellow bellied turtle, inhibition of the heart results. Since the two vagi are the only connections between the medulla and the heart in these experiments, the slowing must be due to the influence of atropine on the brain. This is not due to cerebral inhibition since atropine stimulates the central nervous system. It is due, therefore, to direct stimulation of the cardioinhibitory center of the medulla.

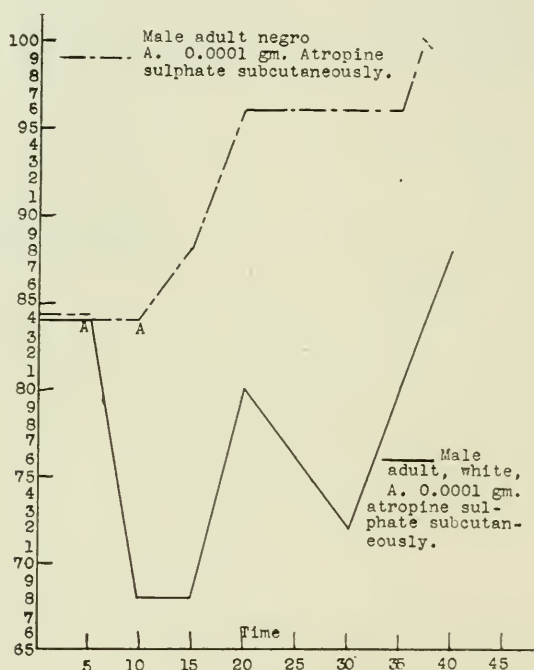


Fig. 7.—Chart showing influence of atropine sulphate on an adult white when given by mouth.

In the dog no inhibition occurs after section of the vagi so that the action is limited to the central nervous system. Likewise, during ether anesthesia no inhibition results. This is probably due to the depression of the cardio-inhibitory center by the anesthetic, atropine, paralyzing the vagus endings before it is able to stimulate the depressed center, as stated by McGuigan.

The action of atropine on the heart rate is determined by the amount of drug used. Apparently the strong drug paralyzes the center for when compared on a time basis, I have found a 0.01 per cent solution to produce inhibition in the turtle more promptly than a 0.02 per cent solution. A 0.002 per cent solution produces a more lasting inhibition, but not so marked or so prompt as a 0.01 per cent. A 0.001 per cent solution is weakly effective, but if perfused for a sufficient length of time may produce inhibition.

Likewise, when large doses of atropine are injected into a dog, inhibi-

tion is generally not produced since the paralysis of the vagus endings prohibits the influence of central stimulation. However, if the atropine sulphate be suspended in olive oil, the absorption is so retarded that the concentration of atropine necessary to stimulate the center is attained, but the amount necessary to paralyze the vagus endings is not reached.

These experimental findings corroborate the results of McGuigan and Harris both of whom obtained clinical evidence that atropine in small doses, 0.001 gm., produces slowing of the heart. With larger doses, acceleration results, due to peripheral paralysis of the vagus.

The action of hyoscine on the turtle brain is similar to that of atropine. Hyoscine, however, depresses the center subsequently, strychnine which I found previously<sup>11</sup> and which Bush<sup>12</sup> later confirmed to be a powerful stimulant of the cardio-inhibitory center, or atropine failing to cause slowing after hyoscine has been used.

#### CONCLUSIONS

Small doses of atropine—0.0003 gram in dogs, 0.001 gram in man produce inhibition of the heart due to direct stimulation of the cardio-inhibitory center.

Hyoscine exerts a similar action to that of atropine on the medulla of *Pseudomys troostii* except that the primary stimulation is followed by depression.

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## BASAL METABOLISM AND BLOOD SUGAR TOLERANCE\*

BY GEORGE HOWARD HOXIE, M.D., F.A.C.P., KANSAS CITY, MISSOURI

THE object of these studies was to determine the clinical value of the methods described; and also to ascertain the relation, if any, between the basal metabolism (thyroid activity) and the carbohydrate tolerance. For the literature on these matters had emanated from research laboratories and large hospital clinics, and it seemed necessary to have a trial of the methods under conditions of actual practice.

The material for these studies consisted of 95 consecutive patients whose condition suggested a study, either of the carbohydrate tolerance or of the basal metabolic rate. Of these patients, eleven were studied more than once. Consequently, we have a list of 106 tests on 95 patients.

The basal metabolic rate was tested on a closed circuit type of apparatus, namely, the Sanborn Benedict, and the Sanborn Handy.

The amount of glucose used was 1.75 grams per kilogram of body weight, according to the suggestions of Janney.<sup>1</sup> The blood was taken at the end of one-half hour, at the end of one hour, and at the end of two hours. The dose of glucose based on body weight seemed more rational than the fixed dose of 100 grams for all weights and sizes of patients, and the two-hour period seemed long enough to determine the type of curve.

The blood was drawn with a 2 c.c. syringe and introduced into a test tube containing a small amount of sodium fluoride and gently shaken to prevent coagulation. The first specimen was taken with the patient in a basal state, 12 to 15 hours after the last food. A specimen of urine was also collected; and then the standardized glucose mixture was given. The second specimens of blood and urine were taken again at the end of the first half hour; the third specimens at the end of the hour after the glucose meal had been given. And the fourth specimens were taken at the end of the second hour. The technic for the determination of blood sugar was the one used with Epstein's Micro-Saccharimeter, as follows:

Two-tenths of a cubic centimeter of the blood is introduced into a standardized tube; then the tube is rinsed with water and diluted up to the 1 c.c. mark. Saturated picric acid is then added—a few drops at a time—up to the 2.5 c.c. mark, shaking the tube gently with each addition of acid. This is then allowed to stand a few minutes, and the contents centrifuged. One c.c. of the supernatant centrifuged fluid is put in a test tube and heated carefully until complete evaporation has taken place. Care is taken not to char the residue. Five-tenths cubic centimeter of a 10 per cent solution of sodium

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<sup>1</sup>Janney, N. W., and Isaacson, V. I. I.: The Blood Sugar in Thyroid and Other Endocrine Diseases, *Arch. Int. Med.*, 1918, xxii, 160.

carbonate is introduced into this tube and gently heated. This is then transferred to the graduated tube used in a small colorimeter and compared with the two standard Tubes A. and B. The solution in the graduated tube is diluted until it matches that in one of the graduated tubes, and the calculation made therefrom.

## GLYCOSURIA

We found 22 patients who showed sugar in their urine under the conditions of this test. The point at which the sugar appeared is shown on the

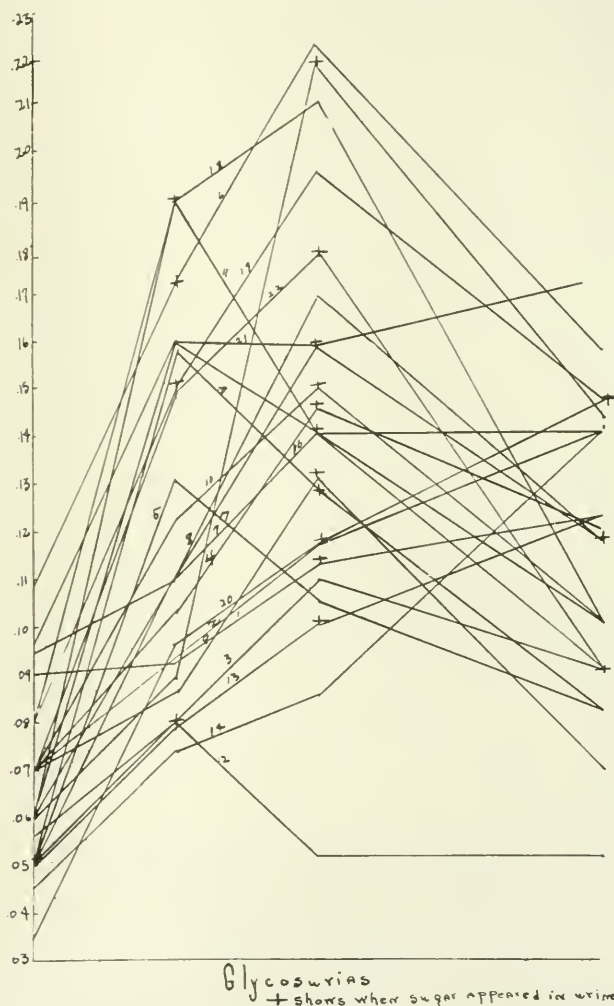


Fig. 1.—Curves of patients whose urine showed sugar during the test. A cross indicates the time at which the sugar was found.

accompanying chart (Fig. 1) by the “+.” This, of course, indicates that the sugar appeared at some point between the two last determinations and should not indicate that the threshold for that individual for that particular amount of glucose was to be found exactly at the point where the “+” is placed. Of



this group, only one had been called a glycosuric before this test, and in that case, the glycosuria was an accompaniment of hyperthyroidism of the adenomatous type (where a preceding colloid goiter had suddenly become active).

Of these 22 glycosurics, eight showed a basal metabolic rate more than 10 per cent above normal. One showed a minus 13 per cent, the rest (13) were within normal limits.

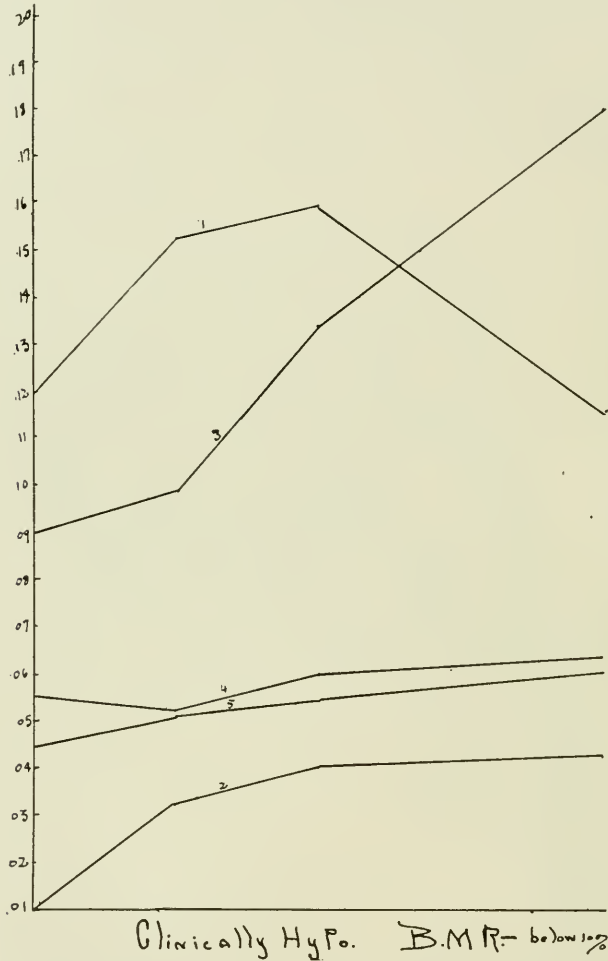


Fig. 2.—Curves of cases in which the calorimeter showed a BMR more than 10 per cent minus.

If we accept as the normal curve for sugar tolerance the return at two hours to approximately the fasting point, and the diabetic curve where the percentage of blood sugar is still increasing at the end of two hours, we find in this group seven diabetic curves. We find only three strictly normal curves; the others show a delayed return to the normal.

The threshold for the appearance of the sugar in the urine is below twelve in six cases. It is interesting to note that only four of the six show the typical diabetic curves; the other two (12 and 9) show the normal curve.

We find, therefore, glycauresis for this amount of glucose in two types of curves: first, those of slow rise and delayed return with the glycauresis occurring below the accepted threshold of 16.5, and, secondly, in those with a quick rise and that to a height above 16.5.

*Hypothyroid Cases. I.*—There were five cases (Fig. 2) in which the basal metabolic rate was more than 10 per cent below normal and in which the clinical symptoms were sufficient to justify a diagnosis of thyroid insuffi-

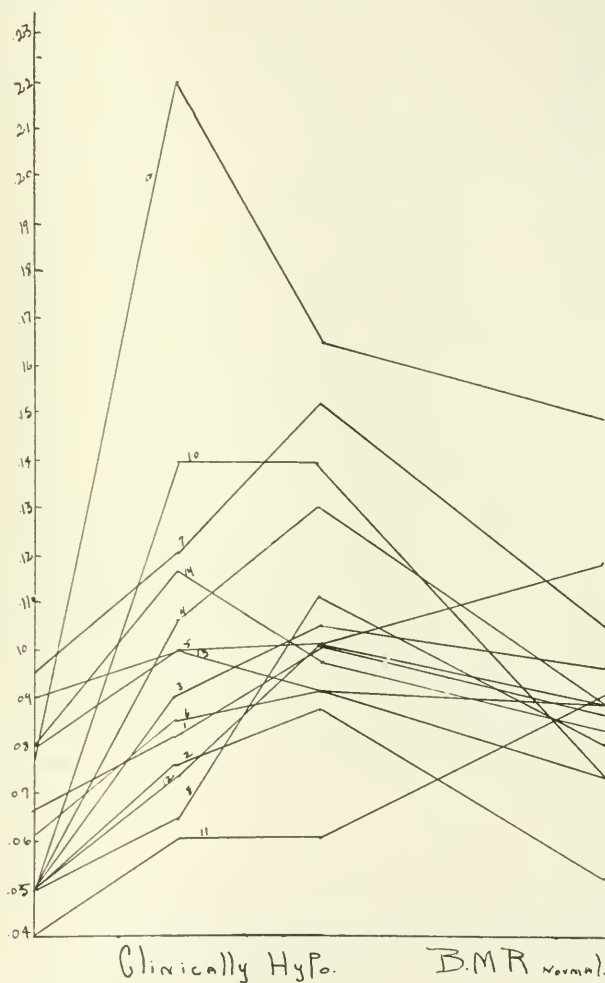


Fig. 3.—Curves of patients with normal calorimetric readings, but where the clinical working hypothesis had been hypothyroidism.

ciency; that is, where there was an agreement between the clinical and laboratory finding of hypothyroidism. It is interesting to note that four of these five cases show typical diabetic curves, but the fifth shows a normal curve in spite of the fact that the fasting sugar percentage is 0.12. One of these cases was studied three times; and after the first study thyroid extract was given. Ten months later, the basal metabolic rate was still slightly below normal but the sugar content of the blood was higher, although the curve

was of the same type. Then the third study three months later was made in which the original low blood sugar content was found again.

*Hypothyroid Cases. II.*—Hypothyroid cases with evidence of pluriglandular involvement. We have three curves, the basal metabolism of all three being lower than minus 10, (-11, -12, -13). The sugar curve in two of them is of the decided diabetic type; and the third is one of delayed return. This group would go toward supporting belief in the parallelism between the

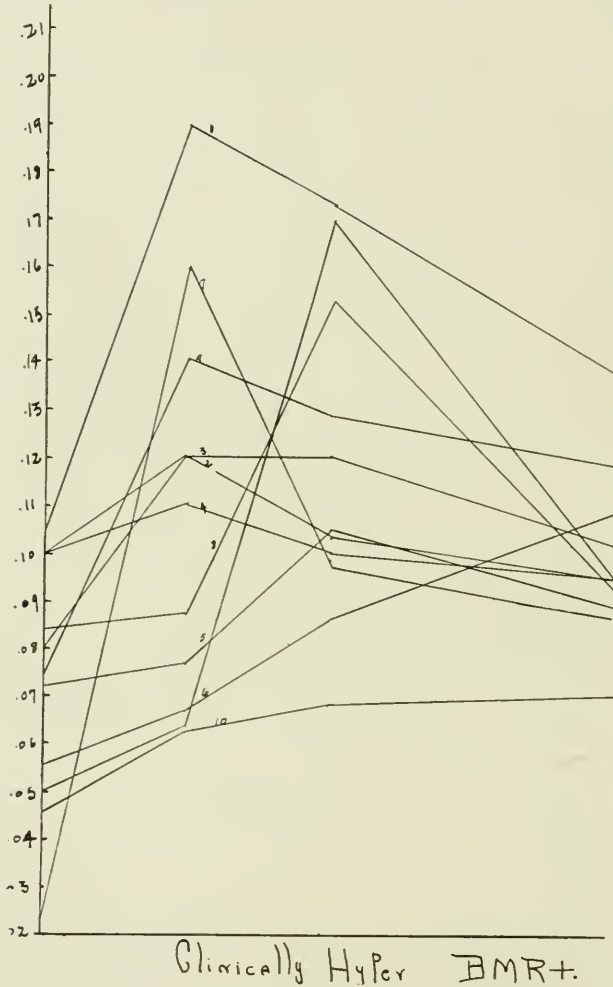


Fig. 4.—Curves in hyperthyroidism.

minus BMR and the diabetic curve. But the unknown factors in these cases were so great that such a conclusion would not be justified.

*Hypothyroid Cases. III.*—Senile Type. Here with a BMR reckoned variously at minus 9 and minus 14, we have practically a normal curve.

*Hypothyroid Cases. IV.*—Focal infections. We have three cases where there seemed to be a disturbance of the thyroid function, the basis for which, however, seemed to lie in focal infection elsewhere in the body. The first of

these, a recurrent stomatitis of the aspergillus type, showed a practically normal sugar curve. The second with a hepatic trouble, having a history of an operation for gall bladder, showed a BRM varying from  $-5.5$  to  $-11$  with a curve almost of the diabetic type. The third with a series of skin infections somewhat more virulent than an ordinary furunculosis with a BMR varying from  $-13$  to  $-6$  showed a curve of the delayed return type.

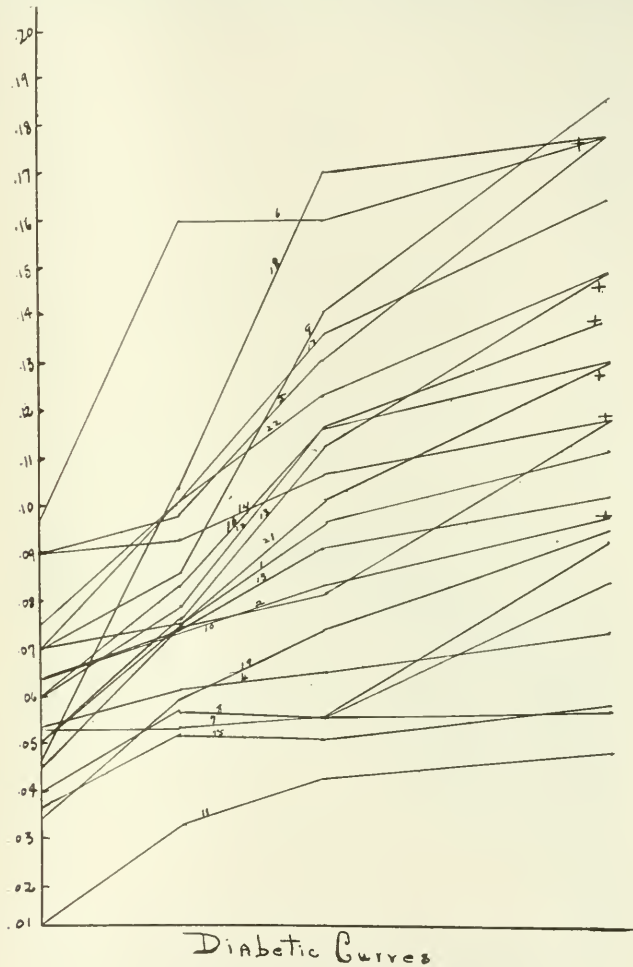


Fig. 5.—"Diabetic" curves.

*Cases clinically hypothyroid, but which showed a BMR within 10 per cent of the normal.*—Fourteen patients came in this class and the sugar curves (Fig. 3) showed two of the so-called diabetic type, four with delayed return, the rest being practically normal. It is interesting to note also that the one case where the sugar curve did not approximate those of the rest of the general group was in a patient where the suspicion is raised that instead of hypothyroidism, the patient may be developing pernicious anemia.

*BMR Normal.*—We had twenty-nine patients whose BMR was within normal limits. In this group, we find one case of an obesity with a  $-9$ ; two cases



of epilepsy, one  $-4$  and the other  $-2$ ; several cases of exhaustion, etc. The curves for the most part are within normal limits, but the few exceptions deserve attention. Of the diabetic curves, one was a case of asthma; another was a case of chronic ulcerative colitis; another was a neurasthenic who would not obey any rules of hygiene; still another was a case of incipient tuberculosis.

*Hyperthyroidism. First:* Cases in which both the BMR was above 10 per cent and there was clinical evidence of the same condition. In this

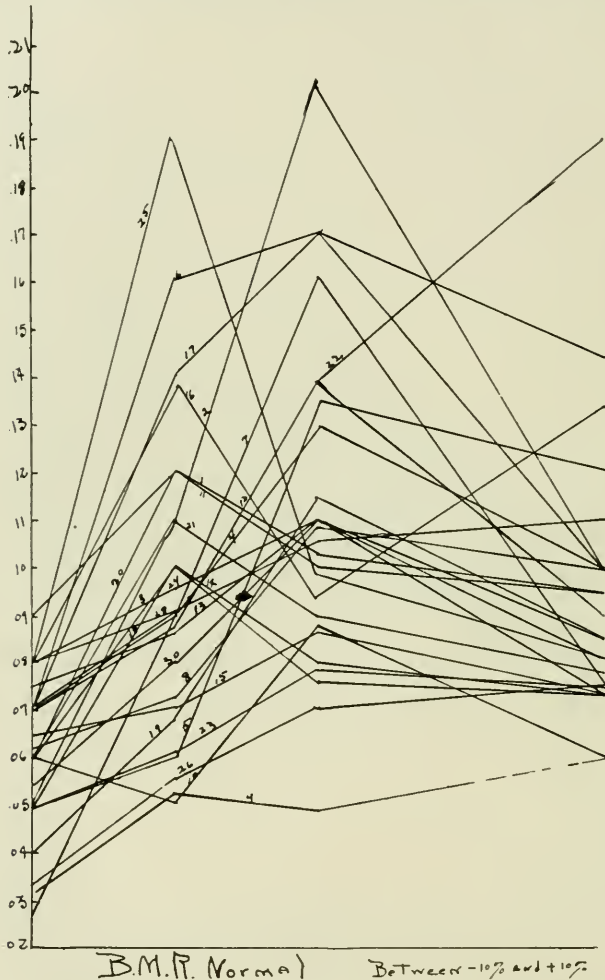


Fig. 6.—Graphs of sugar tolerance in cases wherein the calorimetric reading was within normal limits.

group, there were ten patients whose basal metabolism ran from plus 10 to plus 107. The sugar curves are less uniform than in the groups previously considered (Fig. 4). There were two distinctly diabetic curves, and in both these patients there is clinical evidence in favor of a pluriglandular syndrome.

Thus, a case (No. 7) of pure hyperthyroidism (adenoma) starts in with a fasting percentage of 0.023 and reaches in one-half hour 0.16. This curve is probably as nearly typical of pure hyperthyroidism as any of them; although curve No. 5 is that of another proved case of hyperthyroidism (in which an

operation was performed). The curve is of the same type except for the slow absorption. In this case, the curve starts with 0.05 and reaches 0.17 at the end of one hour. Curve No. 2 was that of a patient treated successfully with x-ray. Here two readings were taken at intervals of a month; and the interesting thing about it is that sugar is taken very slowly into the blood stream. For example, on August 20, 1920, when the BMR was plus 53, the sugar curves were .057, .062, .07, .075. On September 9, the BMR was plus 48,

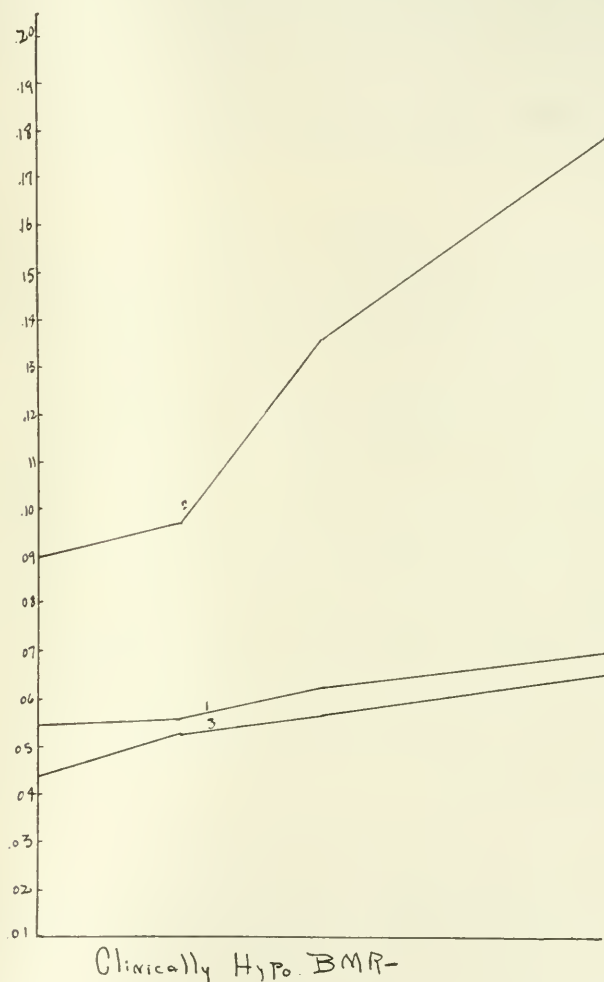


Fig. 7.—Curves of the sugar tolerance in cases wherein the calorimetric reading was also below normal.

and the blood showed .10, .12, .104, .10. It is evident in this case that the condition was not one of severe toxemia in spite of the fact that there was some myocarditis present, for which the patient came originally for treatment.

*Second.*—Cases in which the BMR was above 10 per cent but where the cases clinically seemed to be more neurotic than goitrous. In this group we have six curves, and as might be expected no uniformity. One curve is

extremely low, indicating a hypoglycemia. Another is strictly a diabetic curve. And another is the type with the slow return to normal with a long plateau, such as was marked up by Janney as being due to the loss of the thyroid. In our case, however, there was no evidence of loss of thyroid function either clinically or with a calorimeter.

*Third.*—Cases in adolescents. Of the adolescent group, we have three curves. One patient came in on account of his heart; and his curve shows the sharp rise and the equally sharp fall, while the BMR was plus 22. The second was a boy who was rather dull at school. His curve is nearly normal except that the rise is very slow. This patient was improved by the administration of thyroid extract. The third case was a girl who represented clinically the typically adolescent patient with a prominent thyroid gland (colloid goiter).

#### “DIABETIC” CURVES

We have plotted out nineteen curves wherein the direction at the end of the second hour was still upward (Fig. 5). These fall into a general group with the exception of three curves. The first of these exceptions—where there is a hypoglycemia—has to do with a post-influenzal condition with a minus 20 BMR and considerable pulmonary distress with occasional fever. Here the readings were .01, .032, .04, .045. Another is that of a patient with a history of headache since the birth of her last child, at that time two years of age. The headache was constant but worse when rising from the horizontal position. The blood pressure was exceedingly low but the BMR was within normal limits. The readings were .097, .16, .16, .18. The renal threshold was low and sugar appeared in the urine. The third was a case of exhaustion with the symptoms of duodenal ulcer. Here the BMR was plus 15 and the renal threshold normal. The readings were .047, 0.103, .17, .18.

A most interesting comment on the so-called diabetic curve is that of all of these twenty-two cases showing the typical form, only six showed sugar in the urine during the test.

#### DELAYED SUGAR ABSORPTION

We have four cases of delayed absorption where the angle of the curve at the half hour interval is less than 160 degrees. Three of these show a minus BMR; one shows glycosuria with extreme hyperglycemia. The fourth curve in this group is marked up with a plus BMR, but I believe that that was due to the patient's not being in a basal state. Unfortunately, the test was not repeated.

These results tend toward substantiating the statement often made that delayed absorption of the glucose accompanies decreased metabolism.

#### CONCLUSIONS

1. Our experience would lead us to believe that the simple closed circuit types of calorimeters are worth while in actual practice.

2. The variations in reports on the BMR seem due more to the condition of the patient than to the type of apparatus.

3. Such reports, however, are not final. It is necessary to correlate clinical observation with the laboratory tests.

4. The blood sugar tolerance test cannot be substituted for the calorimeter, nor any close parallelism drawn. Both studies are needed if a complete knowledge of the patient's function and vitality is sought.

5. A revision of the terminology and standards of the blood sugar curves is needed if we are to introduce this study into general practice.



## LABORATORY METHODS

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### A SIMPLE METHOD TO DETERMINE THE COAGULATION TIME OF THE BLOOD\*

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BY HENRY J. GOECKEL, PH.M.D., CRANFORD, N. J.

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EVERY one who has checked surgical cases, especially those to be operated on for nose and throat affections, by determining the coagulation time of the blood, knows the value of this test. It detects cases liable to post-operative hemorrhages if the coagulation time is not materially reduced by appropriate therapeutic means. It enables the surgeon or the attending physician to anticipate trouble and to prevent it or to be prepared to check a hemorrhage should it occur.

On a busy hospital service to employ the Buffi-Brooks or the Brodie-Russell-Boggs coagulometers is time consuming, while the testing of a liberal sized drop of blood on a plain glass slide is open to criticism because the margin of error is greater than is allowable.

The method employed by the writer possesses several advantages for hospital routine use. The equipment is very simple, inexpensive, and is economical in time. This last factor is always an important one in a busy hospital. The blood is not exposed in a large air space and is not liable to desiccation as in other methods.

#### TECHNIC

The finger tip or ear lobe is thoroughly cleaned with alcohol or ether, rubbing it quite briskly with a clean piece of cloth, gauze or cotton to remove as much lymph as it is possible to remove from the area to be pricked, and at the same time to produce a somewhat hyperemic condition.

Three by one inch glass slides, each with two ground-out cavities are used. These are the stock slides used for hanging drop cultures and for the microscopic Widal agglutination tests for typhoid bacillus infections, etc. The cavities are of about fifteen millimeters' diameter and approximately matched.

The time of the appearance of the drop of blood after making the puncture is carefully noted and a rather liberal long drawn drop of blood is placed in each of the cavities of one slide by inverting the cavities over the puncture. This slide is then placed on top of the second slide as is shown in the illustration. This gives enclosed chambers of small size, each with a drop of blood suspended on the upper wall of the chamber. It is not neces-

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\*Received for publication, May 3, 1922.

sary to vaseline the slides to make them air tight if they are matched, neither is it necessary to clip them together if one is reasonably careful.

When the slides are tilted on ends as illustrated, the non-coagulated blood has a tendency to gather toward the bottom border giving a contrast of a light and a dark zone, which effect is accentuated by the slight magnification due to the curved surface of the cavity. This tilting is repeated about every thirty seconds after the third minute, alternating the incline to cause the blood to flow toward the lighter area. When the coagulation begins, a distinct clumping of the cells becomes visible. When the coagulation is completed no mobility will be seen, that is all parts of the drop will become fixed or coagulated.

By this method a coagulation time up to seven minutes appears to be a safe limit.

Since employing this method at Muhlenberg Hospital we have had two hemorrhage cases. One was a known bleeder anaphylactic to serum, who had received lactates without success (thyroid deficient), and another case which by oversight was not tested. This case showed a postoperation coagulation time of eleven minutes.

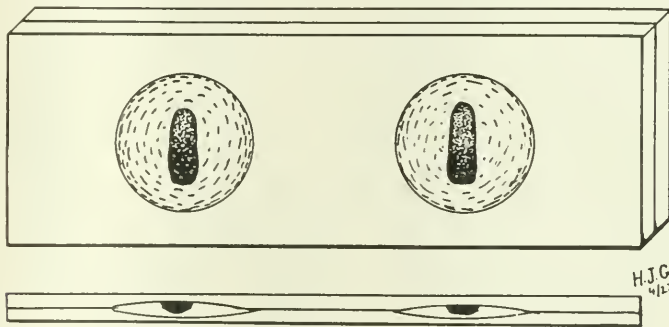


Fig. 1.

#### SUMMARY

The advantages for this method are:

The equipment is simple and inexpensive.

By using a fresh slide for each case, many tests can be made in rapid succession without having to stop to clean the surface of an apparatus each time.

Each blood is tested in duplicate.

There being no air currents or large air space, evaporation is reduced to a minimum.

The curved surface of the cavity being somewhat magnifying, no lens is necessary to observe the changes.

The used slides can all be thoroughly cleaned at one time saving time and material.

The method is satisfactory for routine use. If anything it is somewhat more accurate than the special methods mentioned and is much more accurate than the exposed drop method.

## A MASK FOR THE ALVEOLAR AIR TESTING OUTFIT\*

By HENRY J. GOECKEL, PHM.D., CRANFORD, N. J.

FOLLOWING is a description and illustration of a mask used with the Marriott Alveolar Air Testing Outfit used for the rapid clinical determination of carbon dioxide tension. It was improvised by the writer from material usually at hand in an average hospital.

The material required is an inflatable rubber cushion ring such as is used on the face mask of the Flagg or other anesthesia outfit for gas inhalation; a piece of rubberized cloth from a discarded hot water bottle or a piece of heavy rubber sheeting; a piece of glass tubing flared at one end and tapered somewhat at the other end; a strip of adhesive plaster or rubber

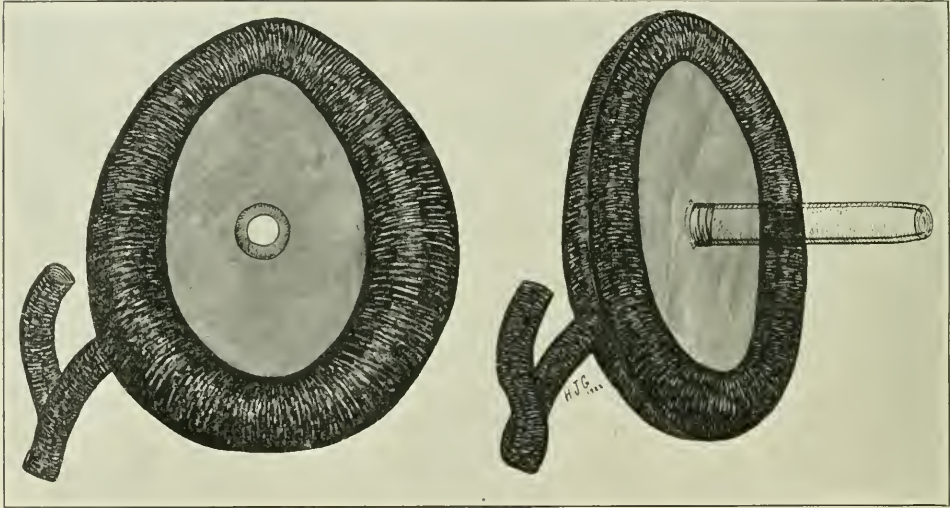


Fig. 1.

tissue; and a suitable rubber cement. The illustration shows how these are put together.

The cushion is well inflated and the side rubber tube is closed with the aid of an elastic band. This gives a masked breathing tip which can be securely placed over the nose and mouth of the patient and can be held firmly in place by the operator without elaborate preliminary adjustments and without discomfort for the patient. The air bag is attached to the glass tip in the usual manner.

Requiring practically no cooperation by the patient except to breathe, it can be employed equally well on conscious and on comatose cases. Being comparatively simple in appearance and comfortable when applied, it is not likely to arouse apprehension in the patient preceding the test. It possesses the important advantage that it can be readily cleaned.

\*Received for publication, May 3, 1922.

## A SIMPLE APPARATUS FOR CONTINUOUS EXTRACTION OF LIQUIDS BY LIQUIDS LIGHTER THAN WATER\*

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THE quantitative extraction of liquids with ether and other solvents lighter than water by means of a separatory funnel is tedious, inaccurate and impractical. This is true of such a fluid as urine which tends to emulsify readily with ethereal solvents and, therefore, quantitative recovery is greatly impaired; and also of large volumes of fluids containing small quantities of solutes. Similar conditions arose in this laboratory in connection with a study of the urinary excretion of volatile and non-volatile salicyl ester compounds. For this purpose an apparatus that could be operated automatically, yet accurately, with large volumes of urine without attention was required. None was available in the market. Therefore, the apparatus described in this paper was devised and found to answer our requirements. It is believed that it is suitable for a variety of fluids and ethereal solvents. The advantages of this apparatus are simplicity, ease of construction and availability and satisfactory operation for quantitative purposes.

The principle employed in this apparatus is continuous, slow percolation of ether through the fluid to be extracted in an ordinary drug percolator. The supernatant layer of ether is automatically drained by an outflow tube which can be set at any desired level, depending on the volume of fluid (urine, etc.), to be extracted. A small, glass stop-cock is attached to the outflow tube for withdrawal of the ether extract to determine completion of the extraction. The percolation tube consists of two tubes and a receiving end for a condenser, all in one piece. One tube has a perforated bulb and descends into the liquid in the percolator and the other is outside the percolator for returning ether from the extraction flask to the condenser, which is attached to the receiving end of the tube. A diagram of the apparatus is presented in the accompanying figure and requires no explanation.

The apparatus is so simple that it can be easily constructed in the laboratory from parts available in the market. The following parts and their dimensions are used in the construction of an apparatus of 500 c.c. capacity: One pint drug percolator with large cork stopper to fit upper end snugly; outflow tube made of glass tubing 3 mm. bore and 31 cm. long from top to bend at neck of percolator, with a small thistle-like enlargement at the upper end 8 mm. diameter. This enlargement facilitates drainage of the ether extract and should be small enough to pass through the rubber stopper in the neck of the percolator. The lower end of the tube is bent at right angles

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at the neck of the percolator and again at the extraction flask, the distance between angles being about 6 cm. Between the angles an ordinary burette stop-cock is fused for withdrawal of ether for testing. The tubing used for the percolation tube is of 1 cm. bore, the straight tube immersed in the percolator being 30 cm. long from the receiving end to the bulb and the bent outside tube being 45 cm. long from the angle and 3 cm. from the angle to the receiving end, which has a cork stopper with one hole for a short Liebig condenser. An extraction flask of 150 c.c. capacity is used. This is joined to the outflow tube and the outside portion of the percolation tube through a 2-hole cork stopper. For an apparatus of smaller capacity the parts are correspondingly reduced in size. In order to avoid use of a large cork in the percolator, this part of the apparatus could be made from large tubing which is constricted to a narrow neck at the upper end. However, I have

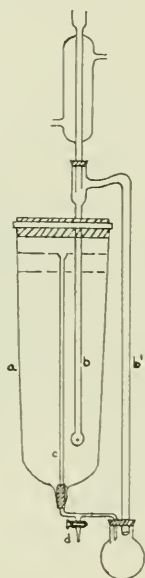


Fig. 1.—Apparatus for extraction of liquids by liquids lighter than water. *a*, percolator; *b*, percolation tube with outside tube, *b'*; *c*, outflow tube with stop-cock, *d*.

experienced no difficulties with percolators, which have the additional advantages of being easily available and cleaned and possessing considerable strength. Leakage of volatile substances through the cork stoppers is completely avoided by applying 15 per cent gelatin over their surfaces and allowing it to harden.

*Operation.*—With the outflow tube in position, the percolator is clamped to an iron stand and the fluid to be extracted is poured in. Then the percolating tube is adjusted to the percolator and extraction flask and the solvent is allowed to flow gently through the percolating tube until about 25 c.c. flow over from the supernatant layer into the extraction flask. Then the Liebig condenser is attached and the extraction flask is placed on the surface of water in an ordinary wash-pan maintained at about 64° C. by a thermo-regulator, using an electric coil immersed in the pan or an ordinary electric

plate for heating. A shallow sand-bath placed over an electric hot plate is also convenient. In this way a battery of 6 to 12 extractions can be operated automatically and continuously without attention. In adjusting the outflow and percolating tubes the following points should be borne in mind: (1) With a given volume of liquid to be extracted, the length of the overflow tube must be such that the open end of the tube is far enough above the surface of the liquid in the percolator to allow for any increase in volume due to solubility of the solvent. With 500 c.c. of urine the increase in volume after continuous percolation with ether from 48 to 58 hours amounts to about 5 per cent (25 c.c.); (2) The percolating tube must be long enough to permit a certain amount of adjustment upward or downward so as to obtain the hydrostatic pressure necessary for passage of the solvent through the holes in the bulb.

An apparatus of 500 c.c. capacity has been tested several times with small quantities of salicylosalicylic acid and acetylsalicylic acid (nonvolatile) and methyl salicylate (volatile) added to 500 c.c. of urine. Table I indicates that the quantitative recovery is entirely satisfactory:

TABLE I

VOLUME OF URINE USED (C.C.)	COMPOUND AND QUANTITY ADDED (GM.)	QUANTITY RECOVERED (GM.)	PER CENT RECOVERY	DURATION OF EXTRACTION WITH ETHER (HRS.)
500	Methyl salicylate 0.10	0.09	99	41
500	Salicylosalicylic Acid 0.2	0.21	98	58
500	Acetylsalicylic Acid 0.010	0.0094	94	55

## CONCLUSION

A simple, convenient apparatus\* for the continuous, automatic and quantitative extraction of nonvolatile (salicylosalicylic acid and acetylsalicylic acid) and volatile (methyl salicylate) solutes in liquids (urine) by liquids lighter than water (ether) is described.

Thanks are due to Dr. Hanzlik for suggestions in construction of the apparatus.

\*The apparatus of different capacities, or any of its parts, is obtainable from Braun-Knecht-Heimann Co., San Francisco, Calif.

## MODIFIED ELECTRODE FOR USE IN CLINICAL ELECTRO-CARDIOGRAPHY\*

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AFTER more than a year's experience with the electrode described by Cohn,† we have found it advisable to introduce a minor modification. Adventure of salt into the crevices between washer, screw, and binding-post, and corrosion of these points of contact gives rise to annoying polarization currents and very high resistance. To assure uniform results it is necessary to remove the binding-post and renew all contact surfaces immediately before making each record. This difficulty has been obviated by changing

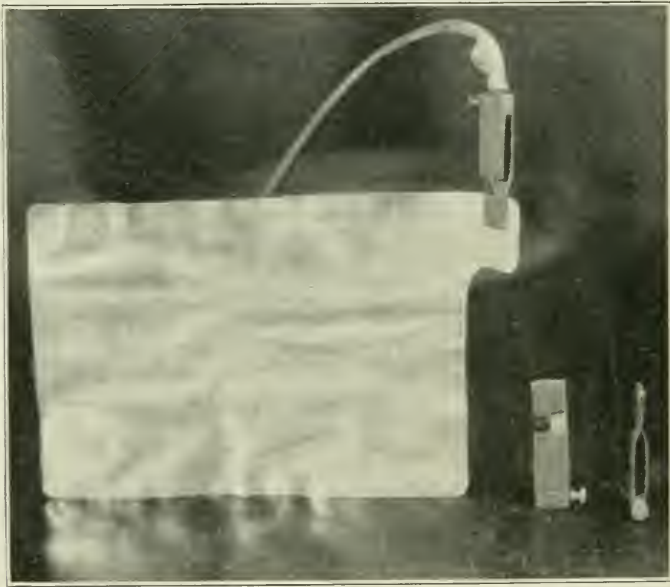


Fig. 1.

the method of making contact. To minimize wrinkling and breaking of the electrode a heavier gauge (B. & S. 21) of roentgen-ray protection foil is used. In cutting the foil a substantial tongue is left at one end. A strong bull-dog clamp, made entirely of brass (gauge B. & S. 17), its jaws slightly turned in at the extreme edge, is applied to this tongue. The base of the clamp is equipped with hole and binding screw which provide for attachment of the lead wire. The essential features of this arrangement are displayed in the accompanying figure. A long, narrow strip of foil, designed to be wound

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†Cohn: Arch. Int. Med., 1920, xxvi, 105.

spirally about the arm, may be substituted for the foil plate shown in the figure. We have dispensed with the original rubber strip covering the metal. The electrode is applied over a cloth soaked in strong salt solution, and is held in place by means of a rubber bandage.

By this device the contact point is usually kept free from salt, but if renewing of surfaces is necessary it may be accomplished without removing the electrode from the limb. No sacrifice of the excellent qualities of the original electrode is involved in this modification.

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## THE ESTIMATION OF URIC ACID IN BLOOD\*

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THE ESTIMATION of uric acid, one of the important products of metabolism found in the blood, is not only regarded as a laborious procedure, but the accuracy of the various methods employed is still considered unsatisfactory by some investigators. Although much work has been done upon the subject since Garrod<sup>1</sup> developed his gravimetric method, little progress was made until Folin and Denis<sup>2</sup> introduced a colorimetric method dependent upon Salkowski's<sup>3</sup> principle of precipitating uric acid as silver urate. The amount of added uric acid recovered by their procedure averaged about 94 per cent. Benedict's<sup>4</sup> modification employing potassium cyanide, not only takes care of the excess of silver, thereby eliminating the hydrogen sulphide procedure, but also intensifies the color produced by the Folin and Denis reagent. This simplification gave a new impetus to research along similar lines. All the above methods, however, require large amounts of blood and concentration of the protein-free filtrate prior to precipitation of the uric acid.

In 1919, Folin and Wu<sup>5</sup> discovered a new blood-protein precipitant, tungstic acid, which yields a filtrate entirely free of proteins. In addition, but a small amount of blood is necessary and the filtrate may be analyzed for uric acid without further concentration. It is important to note in this connection, that they were able to recover quantitatively as much as 20 mg. of uric acid when added to 100 c.c. of blood, thus indicating that no adsorption by the precipitate occurs.

Another method of precipitating uric acid by means of a zinc salt in alkaline solution was suggested by Morris.<sup>6</sup> Recently, Morris and Macleod<sup>7</sup> in conjunction with this precipitant introduced a new reagent arseno-18-tungstic acid, as a substitute for phosphotungstic acid in the analysis of blood uric acid. Another variation which they introduced is the employment of sodium cyanide as the only alkali for development of color. Under these conditions the intensity of the final color produced is approximately three times that obtained by the original method of Folin and Wu.

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\*Contribution from the Dermatological Research Institute. We are indebted to M. Falkov of this institute for valuable assistance in connection with this investigation.  
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Still another modification of the latter procedure has recently been suggested by Pucher.<sup>8</sup> The essential variation consists in precipitating the proteins according to the original method but in addition heating for 10 minutes at 95° C. before filtering. In this way it is claimed, adsorption of uric acid by blood proteins is minimized, as evidenced by more complete recovery of added uric acid. Moreover, the time of filtration of the protein precipitate is greatly diminished.

Benedict<sup>9</sup> introduced a method for the direct determination of uric acid in the tungstic acid filtrate employing a combination of the reagents of Morris and Macleod, and Folin and Denis. The procedure in brief, is as follows: 5 c.c. of the protein-free filtrate are transferred to a test tube, while into another tube is introduced 5 c.c. of the uric acid standard containing 0.02 mg. of uric acid. To each are then added 5 c.c. of water, 4 c.c. of 5 per cent sodium cyanide solution, containing 2 c.c. of ammonia per liter and 1 c.c. of the arsenic phosphoric acid tungstic acid reagent. The contents of each tube are mixed by one inversion and immediately placed in water at 90° C. After 3 minutes, the tubes are immersed in cold water for 3 minutes and read in a colorimeter against the standard.

While engaged in clinical studies involving the daily analyses of many samples of blood for uric acid content, we were attracted to the latter method because of its speed, simplicity and requirement of small quantities of blood. The results varied so distinctly from those obtained by Folin and Wu's method, that an investigation of the limitations of Benedict's method was undertaken.

We proved to our own satisfaction, that, contrary to the suggestion of Pucher,<sup>8</sup> adsorption of uric acid does not take place during the precipitation of proteins by Folin and Wu's method if properly carried out. The following experiment substantiates this statement. Thirty c.c. of sheep's blood was divided into two portions of 10 and 20 c.c. To the 10 c.c. portion was added a solution of uric acid equivalent to 5 mg. of uric acid per 100 c.c. The proteins were then precipitated in each portion and the uric acid determined in the filtrates. Fifty c.c. of filtrate from the 20 c.c. sample of blood were then removed, sufficient uric acid solution added until its concentration in the total volume was equivalent to 5 mg. per 100 c.c. of blood, and the resulting solution analyzed for uric acid. The results are shown in Table I.

TABLE I

URIC ACID IN ORIGINAL BLOOD	URIC ACID ADDED DIRECTLY TO 10 C.C. PORTION	URIC ACID ADDED TO FILTRATE OF 20 C.C. PORTION	URIC ACID RECOVERED FROM 10 C.C. PORTION	URIC ACID RECOVERED FROM FILTRATE OF 20 C.C. PORTION
mgm. / 100 c.c.	mgm. / 100 c.c.	mgm. / 100 c.c.	mgm. / 100 c.c.	mgm. / 100 c.c.
0.0	5.0	5.0	5.0	5.3

The only modification adopted by us was the employment of Benedict's phosphate standard instead of Folin and Wu's sulphite standard. This produced more intense color, thereby permitting more accurate colorimetric

comparisons. In order to prevent adsorption, it is necessary to first add sufficient water so that after subsequent addition of the requisite amount of uric acid solution, the original volume of blood has been diluted by seven volumes of liquid. The precipitation of the proteins is carried out in graduated mixing cylinders. After the 10 per cent sodium tungstate has been added the whole should be thoroughly shaken. The  $\frac{2}{3}$  N-sulphuric acid must be added slowly and with constant shaking to prevent a temporary excess of acid and the formation of clumps of proteins. After all the acid has been added, the cylinder is very vigorously shaken to insure complete mixing. If these conditions have been observed, filtration can be started immediately. We invariably obtain perfectly clear filtrates which are neutral to congo-red paper.

Although, as pointed out by Benedict,<sup>9</sup> uric acid in the concentration existing in the tungstic acid filtrate cannot be precipitated by a silver salt in pure water, we have demonstrated in the following experiment that complete precipitation does occur in the above blood filtrate, due to the presence of other organic substances. After collecting the silver precipitate by centrifuging, the supernatant liquid was decanted and tested for uric acid with Benedict's reagent. It failed to reveal the presence of the slightest trace of uric acid despite the fact that this reagent is much more delicate than any previously used. We also proved that silver urate is completely decomposed by acid sodium chloride, for after treating the insoluble residue a second time with acid sodium chloride, and filtering, the filtrate gave no positive test for uric acid with the above reagent. In view of these facts, it is evident that the method of Folin and Wu permits of a fairly accurate determination of blood uric acid.

A comparison of the two methods was next made using the same protein-free filtrate. The results obtained are indicated in Table II.

TABLE II

NO.	URIC ACID FOLIN AND WU	URIC ACID BENEDICT	NON-PROTEIN NITROGEN
	mgm. / 100 c.c.	mgm. / 100 c.c.	mgm. / 100 c.c.
1	2.2	3.2	35
2	1.9	2.8	36
3	1.8	4.7	31
4	2.6	3.6	40
5	5.2	6.3	47
6	3.0	3.9	33
7	2.0	3.1	42
8	3.2	4.5	33
9	4.0	5.8	55
10	6.2	6.6	44

An examination of the figures shows that higher results were invariably obtained by Benedict's new method. Inasmuch as the Folin and Wu procedure involves many more manipulations than the Benedict method, there is a greater likelihood of analytical error which may possibly account for lower findings. But a series of experiments proved that equally satisfactory recoveries of added uric acid could be obtained by both methods as shown in Table III.

TABLE III  
BY FOLIN AND WU'S METHOD

NO.	URIC ACID IN ORIGINAL BLOOD	URIC ACID ADDED	TOTAL URIC ACID RECOVERED	ADDED URIC ACID RECOVERED
	mgm / 100 c.c.	mgm / 100 c.c.	mgm / 100 c.c.	mgm / 100 c.c.
1	1.9	2.2	4.5	2.6
2	0.6	3.3	3.8	3.2
3	1.3	5.0	6.3	5.0
4	2.3	6.7	9.0	6.7
5	0.0	6.2	6.3	6.3
6	0.0	4.0	3.6	3.6
7	0.0	6.0	5.9	5.9
8	6.2	2.5	9.0	2.8
9	0.0	4.0	3.5	3.5
10	0.0	8.0	8.0	8.0

BY BENEDICT'S METHOD

NO.	URIC ACID IN ORIGINAL BLOOD	URIC ACID ADDED	TOTAL URIC ACID RECOVERED	ADDED URIC ACID RECOVERED
	mgm / 100 c.c.	mgm / 100 c.c.	mgm / 100 c.c.	mgm / 100 c.c.
1	3.2	4.0	7.1	3.9
2	3.7	1.33	5.0	1.3
3	4.1	4.0	7.8	3.7
4	4.1	10.0	13.5	9.5
5	4.4	4.0	8.7	4.3
6	1.2	6.0	7.0	5.8
7	1.2	8.0	8.8	7.6
8	1.2	10.0	11.0	9.8
9	0.0	4.0	4.1	4.1
10	0.0	8.0	8.0	8.0

Since we have already pointed out that no adsorption of uric acid by the blood proteins takes place, and also that the precipitation of silver urate in the Folin and Wu method is complete, we concluded that the higher results found by the Benedict method may be explained upon the assumption that the new reagent produces a color with other blood constituents. We tested the reagent with many organic substances, several of which were similar to those employed by Folin and Denis,<sup>10</sup> with the following results:

1. Aqueous solutions of amino acids such as glycine, alanine and leucine give slight color reactions. With a concentration of acid equivalent to 7 mg. of amino acid nitrogen per 100 c.c., there is obtained a color corresponding to that obtained by approximately 0.15 mg. of uric acid per 100 c.c. of blood.
2. Monohydric phenols containing no amino groups produce no color. Di- and tri-hydric phenols, however, with or without amino groups yield colors increasing in intensity with the number of hydroxyl groups. Thus, with one mg. of resorcin a color equivalent to about 0.04 mg. of uric acid is obtained, while that produced with one mg. of pyrogallie acid is equivalent to about 0.5 mg. uric acid. It is generally conceded that polyphenols are present in the blood.

The effect of these interfering substances, as Benedict has already pointed out, is greatly diminished in the presence of uric acid. Nevertheless there is no doubt that they influence the findings for this substance. Furthermore, the interference decreases with increase in uric acid content.

This can readily be seen upon attempting recoveries with animal blood. The following experiment will serve to illustrate this point. Rabbit's blood was divided into 4 equal parts; in one portion the uric acid was determined directly, the result obtained using Benedict's reagent was 3.7 mg. per 100 c.c. To the other 3 portions were added respectively, 4, 6, and 8 mg. of uric acid per 100 c.c. The total uric acid recoveries were 5.9, 7 and 8.3 mg. Actually, there is practically no uric acid in rabbit's blood, yet this is only indicated in the portion containing 8 mg. of uric acid. The action of the reagent, as Benedict points out, is probably selective; the uric acid reacting more readily than the interfering substances. The above experiment may serve as an explanation of the fact that the results obtained by Benedict's method check with those by Folin's method only in cases of high uric acid content.

Although Benedict has stated that the new method is only applicable to human blood, we found that animal bloods which, according to Folin and Wu's method contained no uric acid, when analyzed by Benedict's procedure, yielded positive findings. Table IV shows the equivalents in mg. of uric acid per 100 c.c. of blood corresponding to the respective intensities of color obtained.

TABLE IV  
URIC ACID BY BENEDICT'S METHOD

mg. per 100 c.c.	
Guinea pig 1	2.2
Guinea pig 2	1.2
Rabbit 1	2.1
Rabbit 2	3.7
Rabbit 3	1.9
Rabbit 4	2.5
Rabbit 5	2.5
Rabbit 6	2.1
Sheep 1	0.0
Sheep 2	4.0

#### CONCLUSIONS

1. Adsorption of uric acid does not take place in the precipitation of blood-proteins by the method of Folin and Wu.

2. Equally good recoveries of added uric acid can be obtained either by the method of Folin and Wu or Benedict.

3. Folin and Wu's method gives correct results for blood uric acid, while the high results found with Benedict's method are due to interfering substances.

4. Benedict's reagent is selective and yields results approximately similar to those of Folin and Wu only with high uric acid content.

5. Benedict's method, owing to its speed and requirement of small quantities of blood, can be used for routine clinical analyses, but is not to be recommended for research purposes.

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## A NEW METHOD FOR STERILIZING PROTEINS AND OTHER COLLOIDAL COMPOUNDS WITHOUT DENATURIZATION\*

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By C. A. MILLS, M.D., PH.D., CINCINNATI, OHIO

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THE preparation of protein solutions for medicinal use internally has always been attended with difficulty and more or less dissatisfaction on account of the inability to completely sterilize the product. This is obviated in part in making serum, antitoxin and thromboplastin preparations by using as nearly as possible aseptic technic in the handling of the materials, the use of only healthy animals, and the addition of a preservative to the final market product. It is realized by all that the cresol, phenol, chloreton or chlora-mine added as preservative will not destroy any spores that should happen to be present, but merely serve to inhibit growth, and possibly kill non-sporogenous organisms. In these protein solutions one is prevented from sterilizing with the more violent chemical reagents, such as formaldehyde, acids, alkalies, or oxidizing agents, because of the sensitivity of the product and the ease with which its physiological value may be destroyed. In making vaccines, tuberculin, etc., heat is used in varying degrees, so that complete sterility may be obtained, but most animal proteins, and, in all likelihood, most bacterial proteins are denaturized by heating above 56° C. in solution, so that we do not know how much more efficient vaccine therapy would be if this possibility of alteration in the proteins was removed.

It was with these points in mind, but more especially with the desire for a method of absolute sterilization of tissue fibrinogen (thromboplastin) solutions for subcutaneous injection, that we were led to devise the method given below.

*Principle.*—This depends on adding  $\text{HgCl}_2$  to the protein solution in sufficient concentration to completely destroy all spores and organisms, followed, after complete sterilization, by the addition of sufficient sterile saturated  $\text{NaCl}$  solution to make about a 12 per cent concentration to decompose the mercury-protein precipitate, and finally the dialyzing out of the soluble double salt, using parchment paper bags and putting copper shavings outside to amalgamate with the mercury as it comes out, thus rendering the process irreversible.

*Method.*—Krönig and Paul<sup>1</sup> determined the time required for complete

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destruction of anthrax spores by varying concentrations of  $\text{HgCl}_2$  finding complete sterility in 12-14 minutes with 1:65 parts  $\text{HgCl}_2$  in watery suspension of the spores, 80 minutes with 1:500, and in 2 hours with 1:1000. For the same effect in protein solutions Behring and Nocht<sup>2</sup> found they must have a concentration of  $\text{HgCl}_2$  of 1:200 for sterility in 80 minutes and 1:1000 for sterility in 24 hours. At various times  $\text{NaCl}$  and  $\text{NH}_4\text{Cl}$  have been added with the  $\text{HgCl}_2$  to prevent precipitation of the proteins, especially in preparations for use in sterilizing sputum and feces, but Krönig and Paul,<sup>1</sup> Scheuerlen and Spiro,<sup>3</sup> and others, showed that the addition of such salts markedly reduced the germicidal value of the  $\text{HgCl}_2$ , presumably through the formation of a double salt in which the mercury was bound in the complex ion.

Taking a solution of tissue fibrinogen containing about 1 per cent coagulable material,  $\text{HgCl}_2$  was added to make a concentration of 1:500, the precipitated coagulant poured into a dialyzing tube and sealed to prevent the entrance of organisms. This was left for 2 hours at room temperature, after which time, sterile saturated  $\text{NaCl}$  solution was added to give a 12 per cent concentration and the tube left standing 15 minutes before immersing in the dialyzing beaker, in order to free all the mercury from the protein. Dialysis overnight at 3° C. with copper shavings in the water outside the tube removed the mercury so completely that the sulphide test failed to reveal a trace in the fibrinogen solution, the protein of which was now entirely back in solution and exhibiting its previous activity. The fibrinogen solution was now found completely sterile, whereas before, although it had stood in the ice box for three weeks after adding 0.3 per cent cresol, it constantly showed the presence of a spore bearing chain bacillus.

*Discussion.*—The author in a previous publication<sup>4</sup> showed that precipitation of the tissue fibrinogen with  $\text{HgCl}_2$  in no wise lessened its activity in quickening blood clotting, after it had been again put into solution by passing  $\text{H}_2\text{S}$  through the suspended precipitate to form  $\text{HgS}$ . Kehoe<sup>5</sup> has recently found, also, that the activity of enzymes may be entirely suppressed by adding  $\text{HgCl}_2$  to their solutions, but that their characteristic activity returns on adding proper concentrations of such salts as  $\text{NaCl}$ ,  $\text{KCl}$ , etc., and that the mercury in these cases becomes dialyzable. Gustav Mann<sup>6</sup> demonstrated clearly many years ago the distinct dissolving action that  $\text{NaCl}$  has on  $\text{Hg}$ -protein precipitates through the formation of the light metal proteinate and the liberation of the mercury.

Although protein substances might be sterilized by  $\text{HgCl}_2$ , then, without permanent loss of their characteristic properties, the removal of the mercury from the solution was a difficult matter. It might be changed to the sulphide, but in the presence of the colloidal protein, this would not precipitate and could not be centrifuged out completely. After adding the  $\text{NaCl}$  to form the soluble and diffusible double salt of mercury, the mercury might be entirely removed from solution by merely immersing copper strips or gauze into the liquid. However, in most instances the salt added to redissolve the mercury would interfere with the use of the sterile product, so

that dialysis is required, either against running water or against a small volume of water with copper present to amalgamate with the mercury. In practice it would be most convenient to use such a volume of water as would leave 0.9 per cent NaCl solution inside the dialysis bag and thus have the sterile product all ready for injection. Such a process is readily carried out in an ice box and we may thus avoid the detrimental effects of exposure of sensitive materials to room temperature.

Other salts might be used with equal effectiveness for redissolving the mercury, all the halides of the light alkaline and alkaline-earth metals and ammonia acting in this way, potassium iodide perhaps working best, but it is simpler and just as efficient to use NaCl and leave the proper concentration in the preparation ready for injection.

It is to be noted that no single step in this procedure is at all new, but the combination of the different steps into a process for protein sterilization without permanent alteration, is new and offers some interesting and important possibilities in regard to the preparation of proteins for injection.

*Summary of Results.*—Protein solutions may be completely sterilized without alterations in their properties by letting stand the required length of time with the proper concentrating of  $\text{HgCl}_2$ , the precipitate being redissolved and the mercury rendered dialyzable by addition of NaCl. The mercury and salt may then be removed by dialysis, placing copper outside the dialyzing tube to amalgamate with the mercury.

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## METHOD OF DIVIDING THE URINARY BLADDER FOR EXPERIMENTAL PURPOSES\*

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BY JAMES A. II. MAGOUN, JR., M.D., ROCHESTER, MINNESOTA

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**D**URING the investigation of various phases of the problem of infection of the urinary tract, the possibility of dividing the bladder into two portions, each in normal relation to its ureter, presented itself.

Female dogs kept under constant ether anesthesia were used. Aseptic technic was observed in all experiments. A low right rectus incision was made and the bladder isolated and packed off with gauze pads. Two stay sutures were placed in each side of the bladder and the organ was divided down its anterior wall into the urethra (Fig. 1). The ureteral openings were

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\*From the Division of Experimental Surgery and Pathology, The Mayo Foundation, and the Section on Urology, The Mayo Clinic.

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identified and a No. 5 ureteral catheter was introduced through each into the pelvis of the kidney. The posterior wall was then divided midway between the ureteral openings as far as the anterior had been divided. A soft rubber

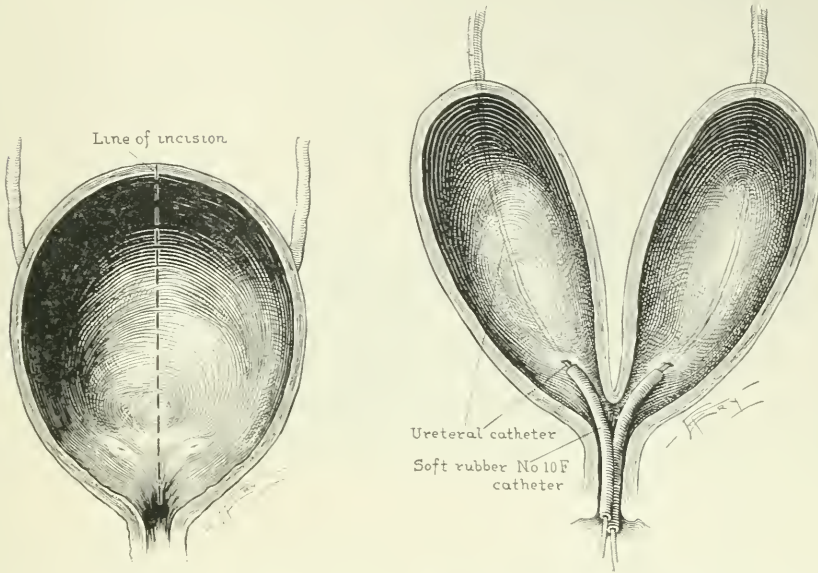


Fig. 1.—Line of incision for division of the bladder.

Fig. 2.—Operation completed. Bladder divided and catheters placed in ureteral openings and urethra.



Fig. 3.—Divided bladder, urethra, ureters and kidneys two months after operation.

No. 10 French catheter was placed into each side of the divided bladder and brought out of the external urethra. The respective ureteral catheters were then led through the urethral catheter to the external urinary opening (Fig.



2). The two segments of the bladder were closed over these catheters with two running sutures of No. 0 catgut. The surrounding fat was sewed over the lower portion of each segment (Fig. 3). The catheters were held in place by a few sutures of catgut just within the labia and were removed in from twenty-four to forty-eight hours. These catheters were advantageous in three ways: (1) as a means of identifying the ureter, (2) as a guide over which to sew the wall of the bladder, and (3) as a means of keeping the newly formed segments of the bladder empty.

The divided bladder permits the study of its cavity in normal relation to the ureter and kidney. Thus, one of the segments may be infected and the changes in the corresponding kidney and ureter may be noted. If obstruction to one segment below the entrance of its ureter is produced, the effect of obstruction at the outlet of the bladder may be studied. It is also possible by this means to study the comparative physiology of the two kidneys. The results of sudden decompression of the chronically distended bladder are now being observed by this method under the direction of the Sections on Urology and Experimental Surgery.

It is suggested that this method of placing catheters may be of some value in resections of the bladder in man.

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### A MODIFIED SAHLI HEMOGLOBINOMETER\*

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BY WILLIAM K. TRIMBLE, M.D., KANSAS CITY, MO.

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**I**N A LARGE hospital where routine hemoglobin estimations are required the method used is of considerable importance. The method must be reliable, quickly executed and free from extrinsic causes of error. Two forms of hemoglobinometers are largely in use, the Tallquist scale and the Dare. Both have a common objection in that the colors to be compared are in shades of red, probably the most difficult shades to compare. The Dare has a fixed and constant light while the Tallquist scale has the disadvantage of having to be read in very variable light conditions. Two workers may make quite widely different readings on the same sample of blood. Again, both are objectionable when a given sample has a brownish shade. The Sahli instrument is not practicable for rapid estimations but the colors to be compared are in the shades of brown. The modified Sahli here illustrated has the advantage of reliability, quick manipulation, simplicity, a fixed light and the reduction of the blood sample to a fixed color.

Select glass tubing of uniform caliber and of about 8 mm. outside diameter. The tubing is broken into 8 cm. lengths and sealed test tube fashion at one end. These are thoroughly cleaned and dried. Ten of these tubes are used in making the scale. A person is selected having a good 100 per cent

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\*From the Laboratory of St. Luke's Hospital.

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hemoglobin. From the vein, without constriction, is drawn one c.c. of blood and placed into a container with a little dry sodium fluoride to prevent clotting. Into a small beaker place 5 c.c. of  $N/10$  HCl and into this place 0.2 c.c. of the blood plus 0.02 c.c. for error. After the blood has completely darkened add 5 c.c. of glycerin. Into the first tube place one c.c. of the hematin hydrochloride glycerin mixture, into the second 0.9 c.c., into the third 0.8 c.c. and so on, decreasing the amount in each tube by 10 per cent, leaving in the tenth tube 0.1 c.c. Each tube is then made up to 1 c.c. by adding  $N/10$  HCl and glycerin mixture. A scale of ten tubes is obtained, the first containing

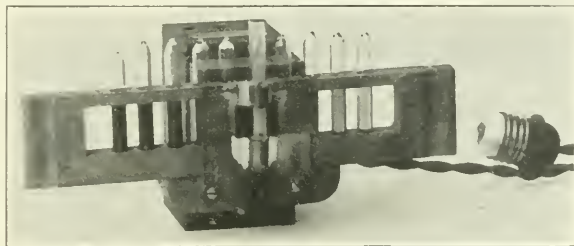


Fig. 1.

20 c.mm. of blood representing 100 per cent and the last 2 c.mm. or 10 per cent. The tubes are sealed in the blow flame. The scale is held in a sliding frame in a rack with a Spencer microlamp fastened to the back.

To make an estimation place 1 c.c. of  $N/10$  HCl into one of the like tubes sealed at the end. Draw 20 c.mm. of blood into the standard Sahli pipette, place into the HCl with repeated rinsings "up and down." When completely darkened place in receptacle in front of the scale and read. As many tubes as needed may be carried in the blood counting tray. It is best to invert the scale daily. If accurately calibrated tubing can be obtained, a more perfect hematin hydrochloride solution may be made but the above is sufficiently accurate for routine work.

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## EDITORIALS

### *Present Status of the Use of Quinidine in Auricular Fibrillation*

SINCE the introduction of quinidine in cardiac therapy in 1918 by Frey, reports of about four hundred cases have appeared in the literature so that sufficient material has now accumulated to draw some conclusions as to the therapeutic value of the drug. Clark-Kennedy has reviewed the work on the first three hundred cases with the following conclusions.

In about one-half of the cases normal sino-auricular rhythm has been restored and in a small proportion of the unsuccessful cases, transient auricular flutter has been produced.

Quinidine acts by prolonging the refractory period and decreasing the conductivity of heart-muscle. During the administration of the drug the auricular rate falls and the ventricular rate rises, due to the lessening of fatigue in the auriculo-ventricular bundle. The transition from fibrillation to normal rhythm takes place usually through impure, and occasionally through pure flutter.

The number of successful cases runs approximately parallel with the dose of the drug employed. Thus Jenny, who gave as much as 46 gr. of the drug per diem, obtained 94 per cent of successes.

The action of quinidine in restoring normal rhythm is most successful in those cases in which auricular fibrillation is of short duration. On the other hand, many successful results have been observed in cases in which the disease was known to have been present for two or three years.

The degree of decompensation influences the action of quinidine unfavorably. But Benjamin and v. Kapff state that the degree of decompensation in which the patient is first seen is of less importance than the degree of compensation to which the patients can be brought by treatment. These authors therefore advise a preliminary course of digitalis in all cases exhibiting decompensation.

If the drug is discontinued, tendency to relapse seems to be the rule, and there is some evidence in the literature that if the drug is continued relapse is less likely to occur.

In the majority of successful cases there is some definite improvement in the objective and subjective clinical conditions of the patient. In many cases there is no change, and in a few cases the patients are definitely worse. In some cases symptoms and signs of failure, which did not disappear under digitalis, have yielded completely to quinidine therapy.

The commonest untoward symptoms of administration of the drug observed, are nausea, anorexia, vomiting, etc. The ventricular rate usually rises and symptoms and signs of increasing decompensation may occur.

Alarming symptoms—sudden pulselessness, cyanosis, apnoea, and unconsciousness—have been observed in a few cases. Frey has reported two such cases. But in neither case did death occur. It is interesting to note in this connection that Schott states that in animals intoxication with quinidine leads to sudden cessation of respiration. Several fatalities are recorded in the literature, but it is difficult to say in these cases whether the cause of death is definitely attributable to the drug. In addition to the two fatal cases reported by Hewlett and Sweeney, v. Bergmann reports two cases in both of which the heart was badly decompensated. One of these died four days after quinidine had been stopped, and the other shortly after a relapse into fibrillation had occurred and when quinidine was again being administered.

Benjamin and v. Kapff report two fatal cases, one of which died of broncho-pneumonia and the other a case fairly well compensated, but which reacted to quinidine with precordial pain and respiratory distress, and died soon after normal rhythm was restored. These authors also call attention to the risk of embolism, reporting one case of pulmonary infarction, and attribute the above case and the alarming symptoms in one of Frey's cases to this cause.

At present there seems to be no relationship between successful restoration of normal rhythm and the etiologic cause of the myocardial disease. All types of cases, rheumatic, degenerative, or associated with exophthalmic goiter, seem to react or fail to react in approximately equal proportions.



Mechanical efficiency of the heart depends upon three chief factors: first, the functional efficiency of the myocardium as a source of kinetic energy; second, the efficiency of the valves in preventing regurgitation of blood and the patency of the valves in offering a minimum of resistance to flow through them; and third, the maintenance of an orderly rhythmic contraction of its component chambers. Interference with any of the three factors may produce a decided handicap in the work of this organ. In cases of fibrillation there usually already exists either myocardial disturbance or valvular disturbance (mitral stenosis) or both. In such cases the reserve cardiac force is already considerably diminished and the advent of fibrillation usually brings cardiac failure. This is of course directly due to the resultant added strain on the heart muscle. Ventricular filling is rendered less efficient by the failure of auricular contraction; there is considerable wastage during the ventricular systole due to the irregular stimulation, and the ventricular rate is no longer controlled by the sino-auricular node.

In certain cases the onset of fibrillation does not bring on heart failure. These are the individuals in whom the myocardium is relatively little affected and the reserve force is still sufficient to care for the added burden, or those who because of previous rest in bed have been carrying a minimal load, or again, those in whom a partial heart block has occurred.

The two methods of treating fibrillation now in use are with digitalis and quinidine. With digitalis therapy a partial heart block is produced so that the weaker auricular stimuli are shut off from the ventricle. A digitalized heart is not functionally efficient because the auricles are still fibrillating and the ventricle remains irregular. The blood is incompletely discharged from the auricles and many of the ventricular contractions are incomplete.

With quinidine a normal rhythm is established, the heart is again dominated by its pace-maker, and the heart rate can be adjusted through nervous reflexes to the rate of diastolic filling and the peripheral blood pressure. Theoretically at any rate, this method of treatment should be more economic of cardiac reserve power.

However, we must bear in mind that this will not relieve the difficulty entirely. Only one of the three factors dominating cardiac functional capacity has been remedied and myocardial or valvular disease usually continues to complicate the picture.

The action of quinidine in fibrillation is best interpreted in terms of Lewis' recent theory of circus movement discussed in a recent editorial in this journal. Quinidine lengthens the refractory period of the auricle from fifty to one hundred per cent. According to the theory of circus movement, lengthened transmission intervals would slow the auricular rate and the longer refractory period would have a similar action, for if sufficiently prolonged, it would divert the wave upon a longer path. Theoretically, normal cardiac rhythm can be restored in flutter or fibrillation by closure of the gap between the crest and wake of the circulating wave. This gap may be closed (1) by an increase in the duration of the refractory period, (2) by an increased rate of conduction, or (3) by a shortening of the path of the circus movement.

As we have seen, quinidine increases the refractory period but it slows the rate of conduction. If these two changes equalize each other, the drug will have no effect in closing the gap. It is only when the former exceeds in amount the latter that closure may be anticipated, and when this does occur, the heart rhythm again becomes regular.

Quinidine acts not alone on the conduction apparatus in the auricle, but also on the vagus nerve with a partial paralytic action, and on the ventricular musculature. This latter action follows diminution of the low grade heart-block which usually accompanies fibrillation and results in an increase in the ventricular rate. Rarely the ventricular rate falls, due to an increase in the P-R interval. Thus we can understand the occurrence of varying responses in different individuals according to which action of the drug predominates.

White points out that chronic heart disease, especially with chronic mitral stenosis and auricular fibrillation of more than one year's duration, is in general a contradiction for quinidine therapy, first because of the danger of embolism when the auricles again start their normal contraction; and second, because in such hearts the rhythm returns to normal only in a small proportion of cases, and even in these there is a decided tendency to relapse. It is in auricular fibrillation of recent origin without heart failure or pronounced mitral stenosis that quinidine therapy seems at present to be especially indicated.

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—W. T. V.

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### *Blood Stains*

THE most commonly used blood stains (of which Wright's stain is the best known example) are combinations of eosin and methylene blue. The precipitate formed when these two dyes are properly combined is dissolved in methyl alcohol in its preparation for use. Before the war, Grüber stains were used for this purpose and the solvent employed was Merck's "reagent alcohol."

Since the supply of foreign dyes was cut off in this country blood stains put upon the market have been very largely unsatisfactory. Some investigators seem to feel the methylene blue at fault and others the eosin; but in light of investigations that have recently been carried on showing the high quality of most American eosins and methylene blues, it would not seem as though the fault could lie primarily with them. There seems more reason to suspect the solvent, inasmuch as Merck's "reagent alcohol" is no longer available. This was such a logical conclusion that two concerns, the Will Corporation and the National Anilin Company, working independently on blood stains, gave special attention to the solvent used, and have prepared

pecially purified methyl alcohols for use in blood stains. Each of these companies at present puts on the market both the blood stains prepared with these solvents and also a methyl alcohol which is indicated on the label to be intended for blood stains. The products of both companies seem to be entirely satisfactory, favorable reports having been received from Dr. J. H. Wright himself.

Although both of these products are satisfactory, special reference must be made to the National Anilin products, because the Will Corporation is not primarily a manufacturer of stains and has in the past been manufacturing only those that they have been unable to obtain in good quality elsewhere. As their policy is to discontinue such manufacture whenever satisfactory sources are located, it is quite possible that they may now discontinue their own blood stains in favor of those of the National Anilin Company. Whatever happens in this particular, however, it is plain that satisfactory blood stains are now available and both of these companies deserve much credit for what they have done in the matter.—*H. J. Conn, Chairman, Commission on Standardization of Biological Stains.*

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# *The Journal of Laboratory and Clinical Medicine*

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## ORIGINAL ARTICLES

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### THE INTERPRETATION OF BLOOD SUGAR ESTIMATIONS THAT ARE NEAR THE NORMAL\*

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BY HENRY J. JOHN, M.D., CLEVELAND CLINIC, CLEVELAND, OHIO

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**W**HEN a case of glycosuria is checked by a blood sugar examination and a value of 125-155 mgm. per 100 c.c. is found, the natural tendency is to disregard this comparatively low figure and to conclude that the patient is a nondiabetic because his blood sugar is so near the normal level. The question is: In such a case how are we to determine whether the individual is normal or is in the early stages of diabetes? Perhaps this problem may be illustrated best by means of actual cases.

**CASE 1.**—A man, aged sixty-two, of Hungarian descent, came to the Clinic with pyonephrosis. There was no diabetes in the family and nothing of especial importance in either family or personal history. Physical examination showed a man in good physical condition with normal blood pressure. His complaint was pain in the loin which radiated down the leg.

**Examination** of the urine showed the presence of albumin, pus and sugar. A functional red test showed an output of phenolsulphonephthalein amounting to 40 per cent the first hour and 20 per cent the second. The Wassermann reaction was negative. Blood sugar examination showed 159 mgms. three hours after a meal and on another day 127 mgms. four hours after a meal. (Within three hours after a meal blood sugar should be normal, i. e., 120 mgms. per 100 c.c.)

Here we have a case with glycosuria which, when tested upon two different days, showed hyperglycemia also. Such a case might be dismissed as one of diabetes but, to establish the diagnosis definitely, a glucose tolerance test was made. In this particular instance 100 gms. of glucose, given on a fasting stomach, showed the following results: (See also Curve 2.)

This curve, with its slow rise and equally slow fall, is distinctly diabetic.

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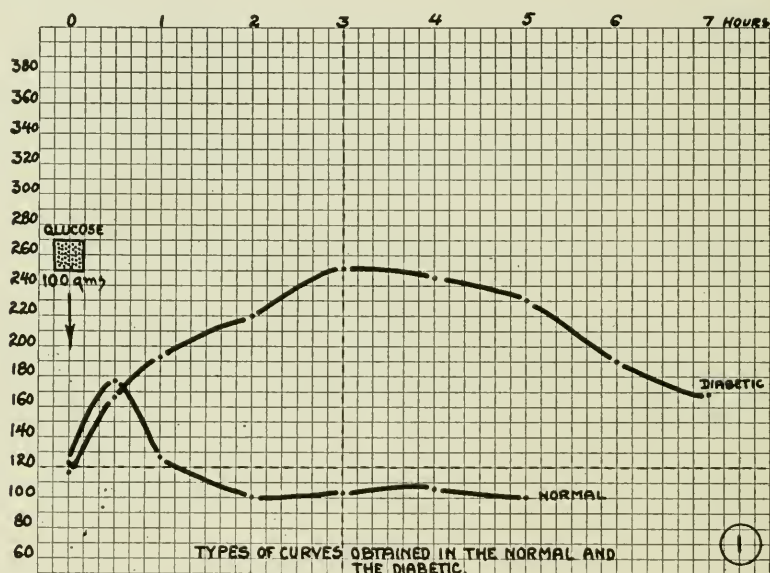
\*Received for publication, July 24, 1922.



## GLUCOSE TOLERANCE ESTIMATION—CASE 1

MGM/100CC.	BEFORE	$\frac{1}{2}$ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400		258	272	330	241	
300						
200						
150	154					133
120						
sugar in urine	trace		plus .26 gm.	2 plus 1.6 gm.	2 plus 1.2 gm.	trace .04 gm.

So the patient was given information concerning his condition and by adhering to a proper diet, which at this stage means only a slight restriction of carbohydrates and no restriction of calories, he should go through life as a mild diabetic.



EXPLANATION OF CHARTS

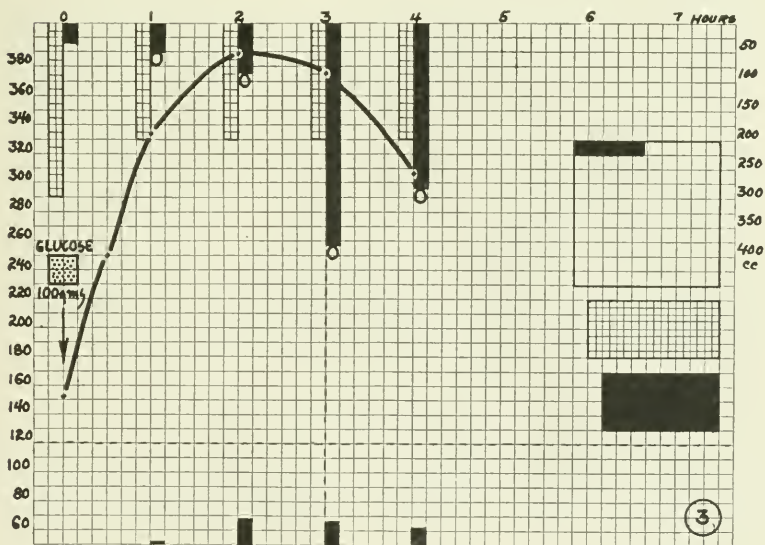
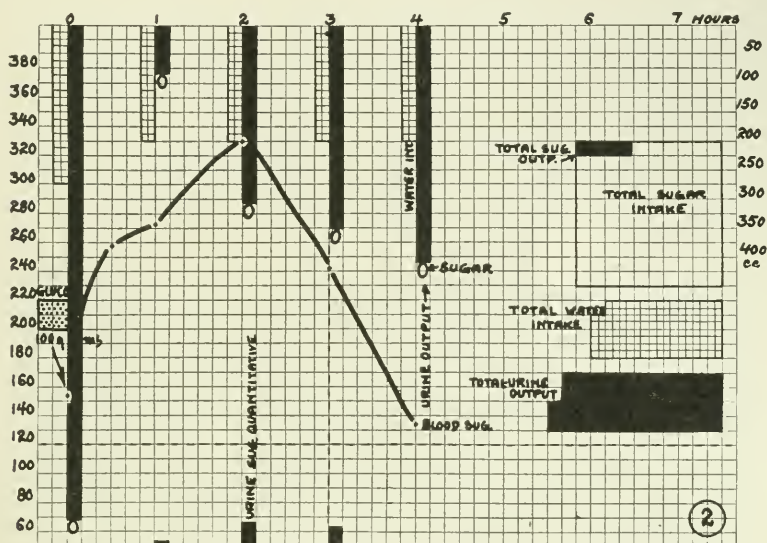
The checkered columns at the top of the charts indicate the water intake; the solid black columns at the top indicate the urine output. The total intake and output during the period of four hours or more is indicated by a like marking at the lower right corner of the chart. The circles at the lower ends of the black columns indicate the presence of sugar in the urine.

The broken horizontal line opposite "120" is the normal level of blood sugar, i. e., 120 mg./100 c.c. of blood. The broken vertical line opposite "3 hours" indicates the period within which, in normal individuals, the blood sugar content again becomes normal after the ingestion of the standard dose of glucose. The heavy curve represents the blood sugar content at the designated periods. The dots which break the glucose tolerance curve indicate the intervals at which blood was taken for sugar estimation. Each solid black column at the bottom of the charts represents the sugar content of the urine output indicated by the corresponding solid black column at the top of the chart. Each square included in these lower columns represents one gram of sugar, and the total sugar output—the sum of these squares—is indicated by the solid black portion of the large square at the right of the chart, which includes 100 squares, representing 100 gms. of glucose—the total sugar intake.

CASE 2.—A married woman, aged forty-three, reported at the Clinic complaining of loss of weight, shortness of breath and hot flashes. There was no diabetic family history and the physical examination was negative. The urine showed: acid reaction; specific

gravity of 1.022; faint trace of albumin, no sugar. Microscopic examination revealed the presence of pus. A blood sugar test, taken on a fasting stomach, showed 166 mgm. of sugar per 100 c.c. and, two days later, 151 mgm. per 100 c.c.

Here we have a middle-aged woman, going through her menopause, who has no sugar in the urine and whose fasting blood sugar is only 151-166 mgm. per 100 c.c. Is this a case of true diabetes or is it one of those excep-



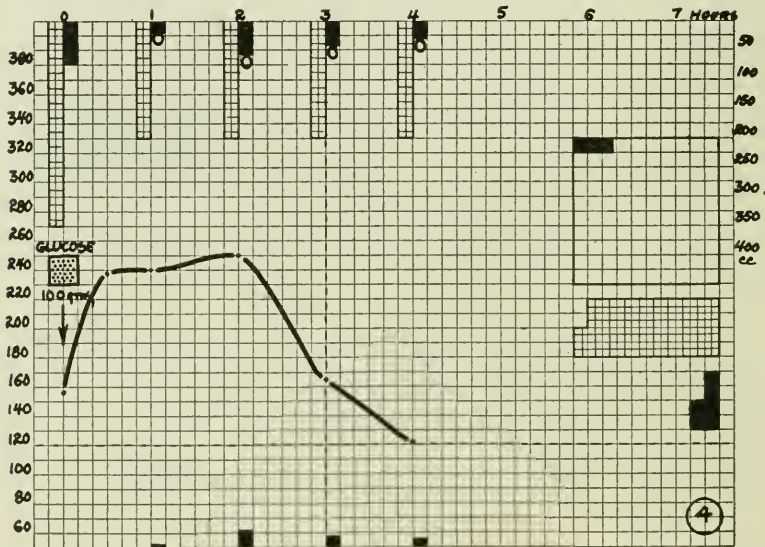
tional cases in which the blood sugar is normally above that of accepted standards? If it were a case of the latter type it would be most unfair to put the patient on a diabetic regimen. Again a glucose tolerance test in this case disclosed the true state of affairs and classified the patient as a diabetic

in spite of the fact that there was no sugar in the urine and the blood sugar was not far above normal. (See also Curve 3.)

#### GLUCOSE TOLERANCE ESTIMATION—CASE 2

MGM/100CC.	BEFORE	$\frac{1}{2}$ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400			333	389	375	306
300		250				
200						
150	151					
120						
sugar in urine	neg.		plus .19 gm.	2 plus 1.87 gm.	plus 1.63 gm.	plus 1.22 gm.

CASE 3.—A physician, aged fifty-six, married, apparently in perfect physical condition, visited the Clinic in order to confirm or to disprove the diagnosis of diabetes which had been made six years previously. He had had measles and mumps in childhood, and typhoid fever



at the ages of seventeen and thirty-five. For the past six years he had been on a restricted diet and wanted to know whether this regimen was warranted in his case.

The urine showed no sugar, was free from acetone and was negative in other respects. The fasting blood sugar was 130 mgm. per 100 c.c. The Wassermann reaction was negative.

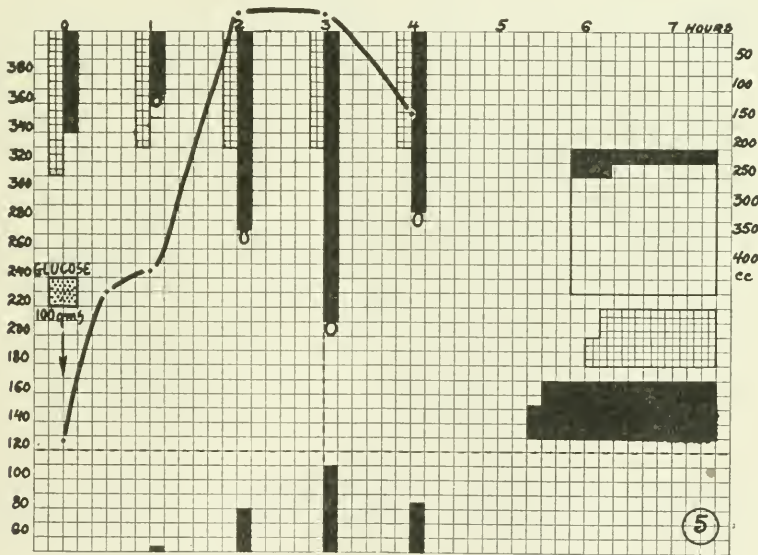
These findings shed but little light upon the justice of the diagnosis of diabetes. Until a glucose tolerance test was made any opinion would have been merely a guess. An incorrect guess, with either resulting laxity or restriction of diet, might either have entailed the breaking down of the patient's carbohydrate metabolism or have forced him to live on foods limited in amount and kind. With the information secured from the glucose tolerance test there was no hesitation about advising the patient that he was a mild diabetic and suggesting that he adhere to a diet moderately restricted as to carbohydrates. (See also Curve 4.)

## GLUCOSE TOLERANCE ESTIMATION—CASE 3

MGM/100CC.	BEFORE	½ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400	156	238	240	250	165	121
300						
200						
150						
120						
sugar in urine	neg.		2 plus .22 gm.	2 plus 1.1 gm.	3 plus .88 gm.	plus 0.6 gm.

CASE 4.—This patient, a clerk—languid, emaciated, pale—was the very picture of a diabetic. The patient was twenty-seven years old, had had scarlet fever at twelve, influenza four years before, several attacks of tonsillitis, and an attack of diphtheria the preceding year. A diagnosis of diabetes had been made two years before by Woodyatt, under whose care the patient had been. He was on the following diet: carbohydrates, 125 gm.; proteins, 45 gm.; total calories, 2,000.

An examination of the urine was negative for sugar and acetone. The blood sugar was 124 mg. per 100 c.c. This normal blood sugar figure in one who presented the physical signs of diabetes and who was on such a high carbohydrate and high fat diet, surprised me greatly. At the patient's request I made a glucose tolerance test which showed the following result: (See Curve 5.)



## GLUCOSE TOLERANCE ESTIMATION—CASE 4

MGM/100CC.	BEFORE	½ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400	126	230	243	428	426	352
300						
200						
150						
120						
sugar in urine	neg.		plus .31 gm.	3 plus 3 gm.	3 plus 6 gm.	3 plus 3.4 gm.

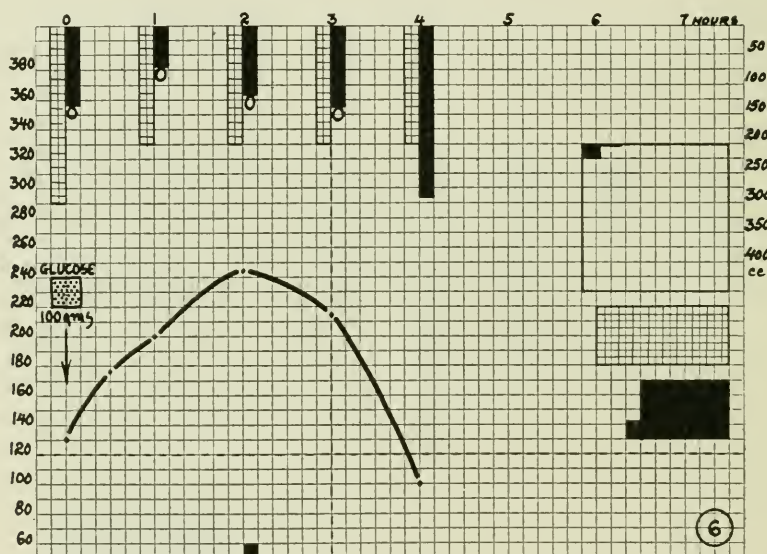
With this typically diabetic blood sugar curve, which by its slow rise and slow fall showed a delay in the utilization of carbohydrates, there could



be no question regarding the diagnosis, in spite of the fact that the patient showed no glycosuria and had a normal glycemia, due to his well calculated diet. His renal permeability was high as can be seen readily from the tolerance chart so he would naturally not show any sugar in the urine unless the blood sugar were quite high. In this case, therefore, we were dealing with a diabetic under control.

CASE 5.—A Jew, aged fifty-five, married, an office worker, complained of stomach trouble and constipation. One sister had diabetes and one brother died of diabetes. There were four children, living and well. The personal history was negative. The physical examination was negative with the exception of a blood pressure of 166/90.

The urine showed a faint trace of albumin but no sugar. After an Ewald meal the gastric contents showed no free acidity and a total acidity of 26. The Wassermann reaction was negative. Fasting blood sugar was 175 mg./100 c.c.; urea, 15 mg.; uric acid, 4.1 mg.; chlorides, 625 mg. per 100 c.c. A few days after the first test, when the patient was not on a diet, the fasting blood sugar was 123 mg. per 100 c.c.



In this instance we had a middle-aged Jew, who had no complaint referable to diabetes and no symptoms of the disease. A blood sugar of 175 mg. per 100 c.c. was somewhat high, yet when this blood sugar was checked by a further observation a few days later, a normal figure of 123 mg. was obtained.

The temptation in this case would be to rule out diabetes on the grounds that there was no glycosuria present and that the blood sugar was normal. Yet, when we went a step further and made a glucose tolerance test we learned that the case was definitely diabetic. (See Curve 6.)

It is with a patient of this type that most can be accomplished for here the ravages of the disease have not yet initiated their destructive processes. The man still had a good carbohydrate tolerance and, by a slight restriction of carbohydrates, could be carried safely and comfortably through life. If treatment were postponed until the disease signs were more marked it would

## GLUCOSE TOLERANCE ESTIMATION—CASE 5

MGM/100CC.	BEFORE	$\frac{1}{2}$ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400						
300				243	214	
200		176	200			
150	130					
120						100
sugar in urine	trace		plus	2 plus	plus	neg.

still be possible to save the patient, but it would be necessary to impose a much more rigid diet which often proves an unendurable hardship.

CASE 6.—A housewife, aged fifty-one, married for thirty-five years, came to the Clinic because she had lost weight, was having hot flashes and ringing in the ears. She had had tuberculosis six years before, rheumatism twenty-five years previously and had had many attacks of gallstone colic. There were three children, living and well. The husband had diabetes but aside from that fact the family history was negative. The physical examination revealed nothing of especial importance. The blood pressure was 140/90.

Urine examination: reaction, acid; specific gravity, 1.040; faint trace of albumin; sugar 4 plus. A functional red test showed 40 per cent phenolsulphonephthalein excreted the first hour, and 14 per cent the second. The Wassermann reaction was negative. The blood sugar was 221 mg. per 100 c.c. A few days later the patient came in without glycosuria and with a fasting blood sugar of 161 mgm., which might have been considered as proof that diabetes was not present if a glucose tolerance test had not clearly told the story. (See Curve 7.)

## GLUCOSE TOLERANCE ESTIMATION—CASE 6

MGM/100CC.	BEFORE	$\frac{1}{2}$ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400						
300		280	204			
200	161			187	171	
150						129
120						
sugar in urine	neg.		3 plus .7 gm.	3 plus 2.7 gm.	3 plus 4.2 gm.	3 plus 1.1 gm.

## SUMMARY

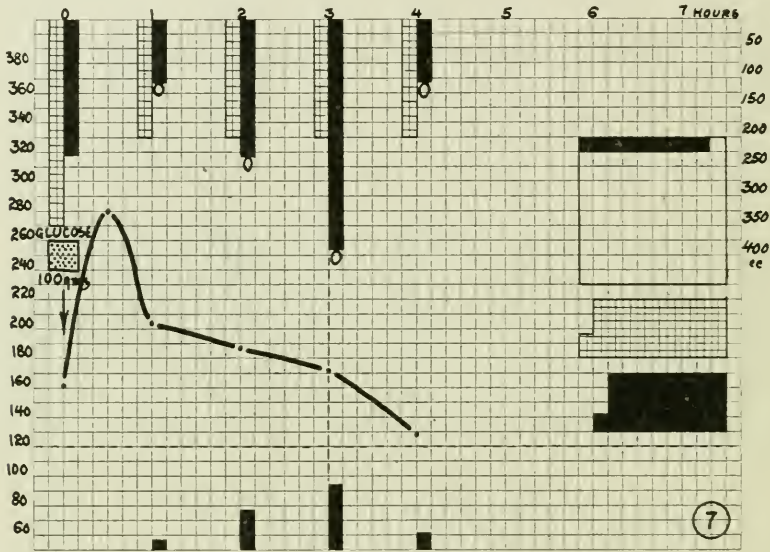
Tabulating the parallel examinations of blood and urine\* in the six

*Case No.	Blood Sugar	Glycosuria
1	127	plus
2	151	neg.
3	130	neg.
4	124	neg.
5	123	neg.
6	161	neg.

cases presented above we make the following observations:

- Glycosuria in one case.
- Blood sugar near the normal in all cases.
- Definite diabetic curve in all cases in which a glucose tolerance test was made.
- Glycosuria in all cases in which the blood sugar level reached the point of renal permeability.

It is clear that we cannot regard a blood sugar level which is near the normal as *normal* unless we know under what conditions it was obtained (whether the individual is on a diet or has been eating sparingly for a time, etc.), and unless we can demonstrate by a glucose tolerance test that the slight rise is of nondiabetic origin. Borderline cases are the most difficult to diagnose and yet the most interesting and the most important. When it is seen that the borderline case has a diabetic trend a simple restriction



of carbohydrates will carry the individual through life comfortably. Usually the total calories need not be changed. On the other hand, if such a case is allowed to drift along without dietary supervision he will reach the stage of diabetes when a *marked* restriction of proteins and total calories, as well as of carbohydrates, will be necessary. In the first instance the man is kept at his work, producing and providing for his family; but if a waiting course is adopted the man is apt to become a permanent invalid and an economic burden.

## OBSERVATIONS UPON THE BLOOD-FLOW IN MAN\*

### II. ESTIMATION OF THE BLOOD-FLOW THROUGH THE HANDS IN CLINICAL CASES

BY N. B. TAYLOR, M.B., ONTARIO, CANADA

IN a previous paper<sup>1</sup> were reported the results of a series of observations upon the blood-flow through the hands as estimated by the calorimetric method of Stewart. The calorimeter, as described in that communication, was designed in accordance with Stewart's original specifications. It consisted of a cylindrical vessel supported in a vertical position and admitted the hand through an opening in its cover. Since there was no provision against the leakage of water between the felt collar and the wrist, it was impossible

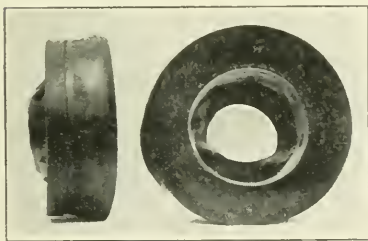


Fig. 1.

Fig. 1.—Showing side and front views of rubber sleeve.

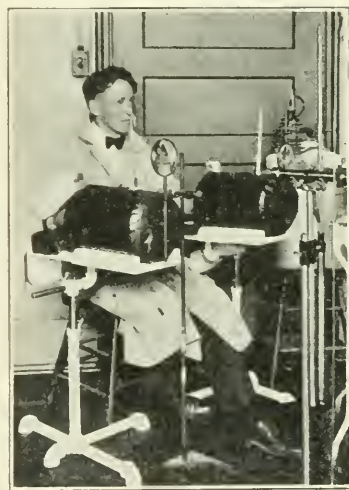


Fig. 2.

Fig. 2.—Showing calorimeters in use in the laboratory.

to employ the instrument in any position but the vertical. This was a great disadvantage in the employment of the method for the estimation of the blood-flow of bed cases and especially of those seriously ill. It was found most difficult to place and maintain the hands in the proper position and on account of the width of the bed, since the apparatus must rest upon the floor on either side, it was impossible to observe the flow in the two hands simultaneously. The construction of the apparatus has been since modified in such a way as to allow of its being placed in the horizontal position without the leakage of water during the observation.

\*From the Physiological Laboratory of the University of Toronto.



## DESCRIPTION OF CALORIMETER AS MODIFIED FOR CLINICAL USE

The calorimeter is similar in general construction to that originally described, consisting of an inner vessel of copper insulated by broken cork from an outer one of galvanized iron. One end of the copper vessel is provided with a flange over which a rubber sleeve is passed and kept upon the stretch. The rubber sleeve which is an adaptation of an appliance previously employed in the treatment of war wounds<sup>2</sup> encircles the wrist and is especially designed, when the hand is in position, to prevent the escape of water. It consists essentially of a flange of thin rubber (Fig. 1) reinforced by

TABLE I  
OBSERVATIONS UPON BLOOD-FLOW BY VERTICAL METHOD

SUBJECT	AVERAGE BLOOD-FLOW FROM A SERIES OF OBSERVATIONS UPON EACH SUBJECT	
	GRAMS PER 100 C.C. OF TISSUE PER MINUTE	
	RIGHT HAND	LEFT HAND
F. G. B.	9.3	6.1
B(a)	8.0	8.0
C. B.	12.0	10.7
B(b)	6.2	6.6
B(e)	12.0	10.0
H. G. C.	11.7	10.8
G. D.	9.4	9.6
H. S. F.		14.1
F.	6.2	5.3
J. E.	9.4	9.8
K. M. H.		4.1
H(a)		8.0
H(b)	6.5	6.7
D. D. H.	8.2	6.7
N. R. H.	8.3	8.5
K. H.	9.5	9.2
A.	4.2	5.4
I. McC.	4.9	7.7
E. M. J.	7.1	6.8
J.	12.6	9.6
J. L.	10.5	8.5
McM.		12.8
M.		7.5
C. N.	6.2	4.8
S. U. P.	6.4	6.3
M. R.	7.5	7.4
R. S.		12.0
S(a)		3.7
S(b)	14.0	10.7
U.	8.8	5.9

Average of entire series of 30 subjects (R. H. 8.6.) Left Hand 8.1.

fingers of thicker rubber which are attached to a thick collar of the same material and from which the thinner flange also arises. The free ends of the supporting fingers are tapered and blend with the thin feather-edge of the flange. Such a construction allows of a perfectly water-tight joint to be established about the wrist and yet does not cause, by undue constriction, any interference with the return of venous blood from the hand. The apparatus may be placed horizontally upon the bed beside the patient without the fear of leakage. Fig. 2 shows the apparatus in use in the laboratory,

a hinged joint allows of its being turned into the vertical position when required.

In preparing to take an observation the water must, of course, be placed in the calorimeter while it is in the vertical position. The hand is then inserted, being passed through the sleeve to a point where the wrist is lightly gripped by the edge of the flange. At the conclusion of the observation the apparatus is turned into the vertical position while the hand is removed and the aperture is closed by a cork plug covered with rubber membrane. The instrument is then returned to the horizontal position while the readings, from which the cooling of the calorimeter is estimated, are taken.

TABLE II  
OBSERVATIONS UPON BLOOD-FLOW BY HORIZONTAL METHOD

SUBJECT	AVERAGE BLOOD-FLOW FROM A SERIES OF OBSERVATIONS UPON EACH SUBJECT GRAMS PER 100 C.C. OF TISSUE PER MINUTE	
	LEFT HAND	
H. J. A.	2.5	
J. H. B.	14.0	
C. S. B.	9.3	
H. E. B.	1.4	
J. B.	8.4	
B.	6.2	
D. G. C.	7.3	
C.	4.3	
D. V. C.	3.9	
D.	7.4	
W. R. F.	11.4	
F.	4.8	
A. H.	1.5	
M. A. H.	8.0	
T. H.	1.3	
J. J.	7.1	
K.	5.3	
J. E. L.	15.4	
McL.	9.3	
M.	8.6	
J. E. M.	12.3	
J. R. N.	5.9	
F. R. P.	11.5	
A. E. R.	9.1	
M. S. R.	6.3	
D. S.	3.8	
W.	6.3	
Y.	9.8	
.....	3.2	
B. R. D.	8.1	

Average of entire series of 30 subjects—Left Hand 7.1.

#### A COMPARISON OF THE RESULTS OBTAINED BY THE VERTICAL AND THE HORIZONTAL METHODS

Since two new features which might have an influence upon the blood-flow, namely, the horizontal position of the limb and the encircling rubber flange, were introduced by the modified apparatus, it was considered desirable to compare the results obtained by the two methods in a large series of cases (Table I and II). It was not possible except in a few cases to com-

pare the results of the two methods when employed on the same subject. The average blood-flow of a group of individuals as estimated by the vertical method has therefore been compared with the average in another group obtained by the horizontal method. There are thirty individuals in each group and several observations over a varying period of time were taken upon each individual. The average of a series of estimations upon each subject has been taken and shown in the columns of the tables. The average of the entire group is shown at the bottom of the respective table. The general conditions of the experiment were approximately the same in the two series. Observations were taken upon the two hands in the case of the vertical method but in the employment of the horizontal method the observations were confined to the left hand. A comparison of the flow through this hand shows higher values for the vertical than for the horizontal method, being 8.1 grams per 100 c.c. tissue per minute in the former and 7.1 grams with the latter method, a difference of about 12 per cent.

#### SUMMARY

1. A modification of Stewart's calorimeter by which the blood-flow may be estimated while the hand is in the horizontal position is described.

2. The results obtained by the two methods are compared. The horizontal method gives values 12 per cent lower than the vertical method.

The author wishes to express his thanks to Prof. J. J. R. Macleod for his help in this research and to Miss Hearn for the preparation of the tables.

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# THE RAPID PREPARATION OF POLYCHROME METHYLENE BLUE STAINS FOR FROZEN SECTIONS OF FRESH AND FIXED TISSUE\*

BY B. T. TERRY, M.D., NASHVILLE, TENN.

## PART I

### FRESH TISSUE STAIN

MANY pathologists are called upon more or less frequently to make very quickly a diagnosis with the microscope of tissue removed surgically. For success in this work a good, reliable, rapidly acting stain is essential. Such a stain can now be made quickly and inexpensively by anyone who is willing to follow carefully a few simple directions.

Before giving my latest method of preparing stains for frozen sections I desire to state that my research on this subject grew out of a visit paid several years ago to the Mayo Clinic in Rochester, Minn. The stained frozen sections shown me there by Dr. William Carpenter MacCarty were much more beautiful and satisfactory than any I had ever seen previously and the technic of staining was wonderfully simple. This stain and technic, I understand, were introduced about 1905 by Dr. Louis B. Wilson whose article on "Staining Sections of Living Tissue, Unfixed"† contains much useful information.

The stain recommended by Dr. Wilson for unfixed tissue is a ripe, Unna's polychrome methylene blue. Formerly, according to Dr. Wilson, the best samples of this stain were obtained direct from Gruebler. During the war when this source of the stain was cut off, it was usually necessary for those using the stain to ripen it themselves. Here a real difficulty presented itself, for the ripening carried out at room temperature was a time-consuming process, requiring 6 months to a year or longer. Moreover many batches of stain spoiled in the ripening so that one was never sure of securing a satisfactory stain even after waiting a year. As this stain required only about 5 seconds for staining, and as the results with it were very beautiful, it was obvious that a more rapid and more certain method of preparing it was desirable.

After a number of experiments I learned how to shorten the time of ripening to 3 weeks and a little later I reduced this time to 6 days.‡ Further work has reduced the time of ripening to a few minutes.

In one of my early experiments I narrowly missed finding the method

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\*From the Department of Pathology, Vanderbilt School of Medicine, Nashville, Tennessee.

Received for publication November 11, 1922. The technic of preparing these stains and the staining results were shown at the Vanderbilt Exhibit at the Southern Medical Association meeting, Chattanooga, Tenn., November 13-16, 1922.

†Wilson, L. B.: Jour. Lab. and Clin. Med., 1915, i, 40.

‡Terry, B. T.: Jour. Am. Med. Assn., 1920, lxxiv, 1775.



I now employ. I had boiled small samples of Unna's alkaline methylene blue stain for varying lengths of time from 5 minutes to 10 hours. Every sample showed plenty of polychrome, but none of them had any strength as a stain. Many temperatures between 37° C. and 100° C. were also tried, but all failed. Months later in studying the details of my experiments I suddenly realized that I had made no tests within the first 5 minutes after boiling began. To find out what occurred in this interval I tested the development of polychrome at 15 second intervals. Much to my surprise I found that I could detect polychrome after boiling for only 15 to 30 seconds, that the polychrome was very abundant and active after 2 to 2½ minutes, but that it was weak and unsatisfactory at the end of 5 minutes. It was obvious that in the first 5 minutes of boiling the stain had ripened and aged.

*Stock Solutions.*—Stock solutions are convenient and save much time if several stains are to be prepared. I use two stock solutions. The first is 100 c.c. of a 1 per cent solution of methylene blue in distilled water. The medicinally pure methylene blue of Merck or Mallinckrodt, the chemically pure stain of the Will Corporation, and other good methylene blues have given excellent results in my hands. The second stock solution is 100 c.c. of a 1 per cent solution of potassium carbonate in distilled water. Merck's U. S. P. potassium carbonate I find excellent. These two stock solutions keep well and will hereafter in this paper be referred to as "Stock Solution No. 1" (1 per cent methylene blue), and "Stock Solution No. 2" (1 per cent  $K_2CO_3$ ).

*Preparation of Stain No. 1, or Alkaline Polychrome Methylene Blue for Fresh Tissue.*—To make one ounce of fresh tissue stain, place in a clean Erlenmeyer flask of 150 to 250 c.c. capacity:

Stock solution No. 1	6 c.c.
Stock solution No. 2	6 c.c.
Distilled water	18 c.c.
	<hr/>
	30 c.c.

Place the flask on a wire gauze supported on a tripod and heat over a Bunsen burner using a flame 6 to 7 inches high. It usually requires heating for about 2 minutes before the solution begins to boil. Boil it exactly 2½ minutes, turn out the flame and, grasping the neck of the hot flask with some convenient holder such as several thicknesses of folded paper, cool the solution quickly at the sink by pouring large quantities of tap water on the outside of the flask. When the solution is cold, pour it into a 50 c.c. graduate and with distilled water make up the stain to 30 c.c. It is desirable to filter this solution into a one ounce bottle through a little absorbent cotton moistened with distilled water and placed in the bottom of a small funnel. The stain is now ready to use. The bottle may be labelled:

"Stain No. 1  
for  
Fresh Tissues."

This stain gives its best results when used on frozen sections of living, unfixed tissue. As soon as the cells die the staining is unsatisfactory.

For detailed directions about the cutting of frozen sections of fresh tissue Dr. Louis B. Wilson's paper already referred to may be consulted. In my experience it is advantageous:

1. To have the tissue to be sectioned as small as is practicable and not thicker than 1 to 2 mm.

2. To embed this tissue thoroughly in ice. This is conveniently done by adding water with a small pipet to the sides of the specimen as the freezing takes place.

3. To cut the tissue as thin as possible consistent with the successful mounting of the sections subsequently. With experience and a good microtome sections 10 to 15  $\mu$  may be cut and mounted.

*To Stain Sections.*—When several good, thin sections have been cut they are transferred to distilled water in a small glass dish over a black background. By means of a needle inserted in a holder one section is drawn up evenly on a slide after immersing the slide in water and getting the section flat on the slide. The section is then covered with 3 to 5 drops of stain which are allowed to act from 3 to 5 seconds. Thick sections may have to be stained longer. With the point of the needle the section is fished out of the stain and transferred to distilled water in a small white porcelain dish or in a small glass dish over a white background. In this dish the section is gently shaken to wash off the excess of stain. While this washing is going on, the stain on the slide is filtered back through moist cotton into the bottle from which it came. The stain is thus used over and over again.

*Mounting the Section.*—The stained section in distilled water is now drawn up for the second time on a clean glass slide, is straightened out, is drained, and is at once ready for examination with the low power of the microscope. For most cases of malignancy this power suffices. If higher powers are to be employed a drop of distilled water on the under surface of a cover glass is lowered gently on the section. Any excess of water may be absorbed with blotting paper or filter paper placed at the edge of the cover.

*Advantages of Stain No. 1 for Fresh Tissues.*—

1. It is quickly prepared. One ounce can be made from the stock solutions in less than 6 minutes.

2. The staining of fresh tissues is very rapid, usually requiring only 3 to 5 seconds. The very short staining period makes it an exceptionally valuable stain where quick diagnoses are important.

3. The cells, nuclei, nucleoli, and mitotic figures are wonderfully sharp and clear. This makes the diagnosis of malignancy easier.

4. The sections can be examined at once mounted in distilled water.

5. As no alcohols are employed the cells are unshrunk and appear larger than they do in sections that have passed through alcohol.

6. The staining solution stains the tissues differentially, nuclei and epithelial cells are blue, connective tissue fibrils red, pink, or colorless, and smooth muscle purplish.

*Disadvantages of Stain No. 1 for Fresh Tissues.*—

1. For the best results this stain must be applied to living cells. If the cells are dead or fixed, they no longer stain precisely. Epithelial cells are more sensitive to deleterious influences than connective tissue and begin to stain diffusely while the connective tissue cells are still sharply stained.

2. The staining solution is not permanent. In the course of weeks or months, depending largely on the weather, the stain becomes overripe or deteriorates and fails to stain intensely. This is not a serious drawback as a new batch of stain can be prepared from stock in less than 6 minutes. As the stain is not permanent it is best to make it up in small quantities. This is the reason my directions are for only 1 ounce.

3. The sections stained are also not permanent. This may be a serious disadvantage especially in difficult cases, for it is not possible to study the tissue long before it becomes unsuitable for further study. See Part II of this paper for a stain which permits long and careful study.

4. The cutting and mounting of unfixed tissue is much more difficult than that of fixed tissue. This is due to the fact that unfixed tissue is so soft and limp that it is much harder to get it flat on the slide for examination.

## PART II

## FIXED TISSUE STAIN

In many instances the tissues I receive for diagnosis are already fixed and with these the fresh tissue stain does not give satisfactory results. Moreover in a large number of cases it is desirable for me to examine quickly many pieces of fixed tissue from autopsies or other sources to determine their suitability for class sections. After working for months with the fresh tissue stain, the older methods appeared to me to be slow and unsatisfactory. It seemed highly desirable, therefore, to modify in some way the fresh tissue stain so that it would stain sharply and precisely tissues that had been fixed. If this could be done the usefulness of the stain would be greatly increased.

On studying the sections of fixed tissue stained with the fresh tissue stain I concluded that they needed clearing. I tested, therefore, a number of clearing agents and found acetic acid was the best. When dilute acetic acid ( $\frac{1}{5}$  per cent to  $\frac{1}{2}$  per cent) was used as a quick wash after staining with Stain No. 1 tissue that had previously been well fixed in formalin, beautiful results were usually obtained. Sometimes the tissues were not washed enough and at other times they were washed too much. In either event the tissues had to be handled a second time and this consumed a little more time. It seemed desirable, therefore, so to combine the acid with the stain that the clearing and staining might take place at the same time. By so doing it was hoped that good results could be obtained more quickly and with greater constancy. Experiments soon showed that this combination could be made.

*Preparation of Stain No. 2, or Acid Polychrome Methylene Blue for Fixed Tissue.*—Pour into a clean Erlenmeyer flask of 500 c.c. capacity:

Stock solution No. 1	20 c.c.
Stock solution No. 2	20 c.c.
Distilled water	60 c.c.
	<hr/>
	100 c.c.

Heat to boiling over a Bunsen burner with flame regulated to a height of about 6 to 7 inches. This preliminary heating usually requires about 5 minutes. As soon as the fluid begins to boil, note the time and boil for exactly  $2\frac{1}{2}$  minutes. At the end of this time turn off the gas and pour cold tap water in large quantities on the flask to cool the contents quickly to room temperature or lower. As the solution cools, the red of the polychrome appears. To the cold solution add 10 c.c. of 10 per cent acetic acid.\* The solution is shaken vigorously for about 1 minute, care being taken not to let it slop out of the flask. It is then poured into a 100 c.c. graduate and with distilled water the solution is brought up to 100 c.c. if, as is usually the case, it is a little under 100 c.c. If it is slightly over 100 c.c., you may not have boiled sufficiently vigorously, but you will probably find the solution satisfactory. Filter into a 4-oz. bottle through a small funnel in the bottom of which is a little absorbent cotton moistened with distilled water. The stain is ready for use. Label it:

“Stain No. 2  
for  
Fixed Tissue Sections.”

The total time of preparation from stock solutions is usually about 10 minutes.†

*Cutting, Staining, and Mounting.*—The technic of cutting, staining, and mounting fixed tissue is similar to that for fresh tissue already described, but is much easier. Fixed tissue is somewhat stiffened so that thin sections are easier to cut and to mount. Sections of well fixed tissue 10 to 15  $\mu$  thick usually require only 5 seconds to stain and are thin enough for ordinary purposes. After washing in distilled water they are remounted in distilled water on slides just as has already been described for fresh tissue. The entire time for staining, washing, and mounting a good section varies usually between 30 and 45 seconds.

*Advantages of Stain No. 2 for Fixed Tissues.*—

1. It is quickly made and keeps well.
2. It acts best on tissue well fixed in formalin.
3. It is precise and rapid, requiring usually only 5 seconds for staining. This makes it especially valuable for the quick diagnosis of surgical tissues.
4. It is excellent in differentiating well fixed tissues,—epithelial cells and

\*One c.c. of glacial acetic acid may be used instead, but the 10 c.c. of 10 per cent acid are more agreeable to deal with and measurements are for this reason more apt to be accurate. A 1 c.c. Luer syringe graduated in hundredths for tuberculin is a convenient instrument with which to measure out accurately 1 c.c. of glacial acetic acid.

†After I had succeeded in combining acetic acid with my fresh tissue stain I found in the literature that Goodpasture, Jour. Am. Med. Assn., 1917, lxix, 998, had already described a somewhat similar method of preparing a good acid polychrome methylene blue stain for fixed tissue. His stain requires about an hour to prepare and with his stain Goodpasture recommends staining sections 1 minute. This is much longer than I find necessary either with his stain or with mine.



nuclei staining blue, connective tissue fibrils red, pink, or colorless, and smooth muscle purplish.

5. It stains nuclei, nucleoli, and mitotic figures with intensity and sharpness. This makes the diagnosis of malignancy especially easy.

6. It stains well and quickly good tissue that has been properly fixed, sectioned, and preserved subsequently for weeks or a few months in formalin. Such sections can easily be used as class material in teaching pathology and histology.

7. A good section after staining can be decolorized in dilute acetic acid, preserved in formalin, and may then be successfully restained with this stain weeks or months later.

The main disadvantage with Stain No. 2 is that the sections stained with it are not permanent. If this drawback can be efficiently overcome, the value of the stain will be tremendously increased.

*Simultaneous Preparation of Stains 1 and 2.*—If one desires to prepare the alkaline and acid stains at one boiling this can be done easily. Measure into a clean 500 c.c. Erlenmeyer flask:

Stock solution No. 1	25 c.c.
Stock solution No. 2	25 c.c.
Distilled water	75 c.c.
	<hr/>
	100 c.c.

Boil exactly  $2\frac{1}{2}$  minutes, cool quickly under the tap, and remove 25 c.c. which are filtered through moist cotton into a bottle. Label it, "Stain No. 1 for Unfixed Tissue."

To the remainder of the stain in the flask 10 c.c. of 10 per cent acetic acid are added, the flask is shaken well, and then the contents are made up to 100 c.c. with distilled water. The solution is filtered through moist cotton into a bottle and is ready for use. Label it, "Stain No. 2 for Fixed Tissue."

*Useful Points.*—1. The amount of acetic acid added to the fresh tissue stain is very important. The quantity recommended is that which seems best for general purposes. If less acid is added the connective tissue fibrils stain more intensely and show more red. If more acid is used the connective tissue fibrils stain less intensely and may be almost colorless so that the epithelial cells stand out prominently by contrast.

2. The time the solution is boiled is very important in developing polychrome. Boiling for 2 to  $2\frac{1}{2}$  minutes seems to be best although good acid stains may be made from solutions boiled only 30 seconds or as long as 5 minutes or more. If the alkaline stain is boiled longer than  $2\frac{1}{2}$  minutes or if it is slowly cooled, more red is present, more crystalline precipitate forms, and the stain is less intensely active. With continued boiling the alkaline stain finally loses practically all staining power. The addition of acetic acid in proper amount restores this power by getting the active stain back in solution.

3. An emergency stain can be made in less than 5 minutes by placing in a good sized test tube:

Stock solution No. 1	2 c.c.
Stock solution No. 2	2 c.c.
Distilled water	6 c.c.
	<hr/>
	10 c.c.

Boil this 2 to 2½ minutes, cool under the tap, and the solution is ready to use on unfixed tissue. If you wish to convert it into an acid stain, add 2 drops of glacial acetic acid, shake, and the stain is ready to use. The proportion of acid to be added to the fresh tissue stain is roughly 1 drop of glacial acetic acid to 5 c.c. of stain.

4. Artificial light, unless well corrected with daylight glass, makes the polychrome stains look redder than when examined by daylight. A good artificial light is to be preferred to poor daylight. It is more intense, more constant, and with it more detail is brought out in the sections. A strong incandescent electric light is excellent for determining the amount of polychrome in bottles of stain. Shake the bottle and in a somewhat darkened room view a thin film of the stain by transmitted yellowish artificial light reflected from a white background.

5. *Quick Diagnosis*.—When the patient is on the table under a general anesthetic and the surgeon wants a diagnosis before proceeding with the operation, the pathologist who has on hand or makes quickly both the fresh tissue stain and the fixed tissue stain, has two courses before him. Either he may freeze the unfixed tissue, section, and stain it with Stain No. 1, or he may place a small, thin, selected piece of tissue in a test tube ⅓ full of a 4 per cent formaldehyde solution (10 per cent of the commercial formalin) and may boil it for about 1 minute, cool it quickly in a large amount of cold water, freeze, section, and stain with Stain No. 2. Thin sections thus stained are usually quite adequate for diagnosing malignancy although not so beautiful\* as sections of well fixed tissue. While the second method is seemingly about 2 minutes longer than the first, it may actually be shorter, for it is easy to lose 2 minutes in trying to mount limp sections of unfixed tissue. In spite, therefore, of the time taken to fix the tissue, the diagnosis by the second method can usually be made in less than 5 minutes after the tissue is received by the pathologist. Personally I prefer the second method, although at times I use both. Until you know which method you prefer it might be well to have an assistant boil up several pieces of selected tissue while the unfixed tissue is being frozen, sectioned, and stained. As soon as the microtome is not in use, the boiled sections can be cut and stained with Stain No. 2. A ready comparison of the two methods can then be made and the more satisfactory one chosen.

6. *Test Objects*.—Not all tissues are equally good as test objects. The best tissues I have found for showing the value of the polychrome stains are squamous-celled carcinomata of the cervix uteri and carcinomata of the breast, but other tissues are probably equally good.

7. *Fixation*.—The degree of fixation of the tissue affects the result

\*The problem in rapid diagnosis is not the staining of the sections, but how in a few seconds to fix tissues well. On this problem I am still working.

markedly. At times some sections from a block of fixed tissue stain beautifully. Others from the same block stain poorly. Blocks of tissue are often cut too thick or are put into too little formalin to fix well. Be sure your tissue is good before you condemn the stain. Sometimes letting the sections remain for a short while in distilled water improves the staining. Necrotic tissue and imperfectly fixed tissue will stain poorly with the best of stains.

8. It is very difficult to mount without wrinkles sections of tissue on slides that are at all greasy. Thoroughly flaming both sides of slides that are supposedly clean gets rid of all traces of fat.

#### AN OFFER

If any one tests carefully the directions here given for preparing Stains 1 and 2 and yet fails to get good results, I will mail that person free of charge a small sample of good acid stain and a few fixed sections that should stain beautifully, provided that within three months after this article is published that person will send me his name and address and will tell me what difficulty he has had. I may be able to set him right.

#### ACKNOWLEDGMENTS

To my assistants and technicians past and present my cordial thanks are here expressed for the interest they have shown in my experiments and for the substantial aid they have given me.

THE ACTION OF MORPHINE, CODEINE, AND APOMORPHINE AS  
SHOWN BY PERFUSION OF THE MEDULLA OF THE  
TERRAPIN (PSEUDOMYS TROOSTI)\*

BY W. J. R. HEINEKAMP, CHICAGO, ILL.

WHILE morphine is characterized by its depressing action on the higher functions, McGuigan and Ross<sup>1</sup> have found that subarachnoid injections of morphine produce convulsions similar to those of strychnine. They believe this action is due to oxidation products of morphine, since morphine treated with nitric acid and then neutralized produces spasms more quickly when injected than morphine *per se*. They proved the site of action to be the cord. Tamura<sup>2</sup> confirmed the work of McGuigan and Ross and stated that the convulsant action is due to 2-nitrosomorphine which he prepared in a pure state by the action of nitrous acid on morphine. In his second article<sup>3</sup> he states that the stimulation in morphine poisoning is due to the oxidation products of mor-



Fig. 1.—Inhibition produced by perfusing medulla with 1-10,000 morphine sulphate.

phine. Jackson and Ewing<sup>4</sup> state that morphine increases the reflex excitability of the vagus center, a fact which I later<sup>5</sup> confirmed. The stimulating power of morphine is established, and recently Bush<sup>6</sup> found that morphine when perfused through the medulla of the terrapin stimulated the cardio-inhibitory center.

Codeine, like morphine, exerts a mixed action of depression and stimulation, but while it is stated to have more tetanic action, its narcotic influence is less than that of morphine. While it has been definitely proved<sup>7, 8</sup> that morphine is oxidized in the body, codeine apparently is not changed. However, if codeine be oxidized *in vitro*, neutralized and then injected intrathecally, it produces strong spasms, which have been proved not to be due to the added factors or to excess acid or alkalies. Codeine when injected into frogs produces spasms more quickly than does morphine.

Apomorphine is characterized by its stimulating action on the vomiting center,<sup>9</sup> although small doses may produce hypnosis; and in animals incapable

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of vomiting a period of excitement is noticed. Solutions of apomorphine turn dark green on oxidation and apparently lose some of their potency.

In this series of experiments I have found that morphine sulphate stimulates and sensitizes the cardio-inhibitory center, later depressing it. When morphine is partially oxidized with nitric acid and then perfused, this action is more pronounced and occurs more quickly. Apomorphine hydrochloride in



Fig. 2.—Inhibition produced by perfusing medulla with 1-10,000 apomorphine hydrochloride.



Fig. 3.—Inhibition produced by 1-10,000 oxidized morphine hydrochloride.

fresh solution first stimulates, then depresses the medulla, known stimulants failing to act after it has been used. Old solutions of a dark green color have no effect. Codeine phosphate exerts no influence *per se* when perfused through the medulla, but when oxidized with nitric acid it exerts a more powerful stimulating action on the cardio-inhibitory center than either morphine or apomorphine.

## EXPERIMENTS

In the following typical experiments all the solutions used were 0.01 per cent in strength except where noted. The methods used were those employed in previous experiments.<sup>10</sup>

## EXPERIMENT I

TIME	HEART RATE	REMARKS
—	48	Normal
11:10	48	Perfusion of medulla with codeine phosphate started.
11:30	44	Slight slowing; return to normal.
11:32	0	Vagus stimulation.
11:37	46	Perfusion of medulla with morphine sulphate started.
11:40	—	Head reflexes active.
11:42	0	Inhibition complete; return to normal with Ringer's.
12:00	36	
12:20	45	
12:25	46	After perfusion with Ringer's.
12:26	10	After perfusion of medulla with apomorphine hydrochloride.
12:30	38	After perfusion with Ringer's.
12:42	22	After perfusion of medulla with morphine sulphate.

## EXPERIMENT II

TIME	HEART RATE	REMARKS
2:22	26	Normal.
2:32	0	After perfusion of medulla with apomorphine hydrochloride. Return to normal with Ringer's very slow. Slowing still evident at 2:50. Vagi active.
2:56	25	After perfusion with Ringer's.
3:15	25	After perfusion of medulla with emetine hydrochloride.
3:50	17	After perfusion of medulla with apomorphine hydrochloride.

## EXPERIMENT III

TIME	HEART RATE	REMARKS
2:15	39	Normal.
2:27	39	After perfusion with morphine sulphate; reflexes.
2:35	39	Very active after perfusion with Ringer's.
2:35:10	0	After perfusion with 2-nitroso morphine; reflexes active.
2:50	39	After perfusion with Ringer's.
3:03	22	After perfusion with morphine sulphate.
3:03:20	0	After perfusion with 2-nitroso morphine.

## EXPERIMENT IV

TIME	HEART RATE	REMARKS
2:20	40	Normal.
2:35	0	After perfusion of medulla with morphine sulphate; head reflexes exaggerated.

## EXPERIMENT V

TIME	HEART RATE	REMARKS
10:25	28	Normal.
10:30	28	Perfusion of medulla with codeine phosphate started.
10:55	27	After perfusion of 100 c.c. of codeine phosphate.
11:20	28	Ringer's perfused since 10:56.
11:22	28	Oxidized codeine 0.005 per cent started through medulla.
11:24	14	
11:27	10	After perfusion of 5 c.c. of oxidized codeine phosphate. Ringer's solution started.
11:30:40	0	Total inhibition lasting until 11:51. Recovered.

## DISCUSSION

The above experiments indicate that the various numbers of the opium group of alkaloids exert different influences on the medulla of the yellow-bellied terrapin (*Pseudemys troostii*).

Morphine stimulates the cardio-inhibitory center as evidenced by the slowing of the heart. It also sensitizes that portion of the central nervous system exposed to it as indicated by the aggravated reflexes. This latter action is similar to that of strychnine and is in accordance with the findings of McGuigan and Ross. It is apparent from their work as well as that of Tamura and from Experiment III that the stimulating action of morphine is due to oxidation products since morphine treated with nitric acid effects these changes more rapidly and strongly than does untreated morphine.

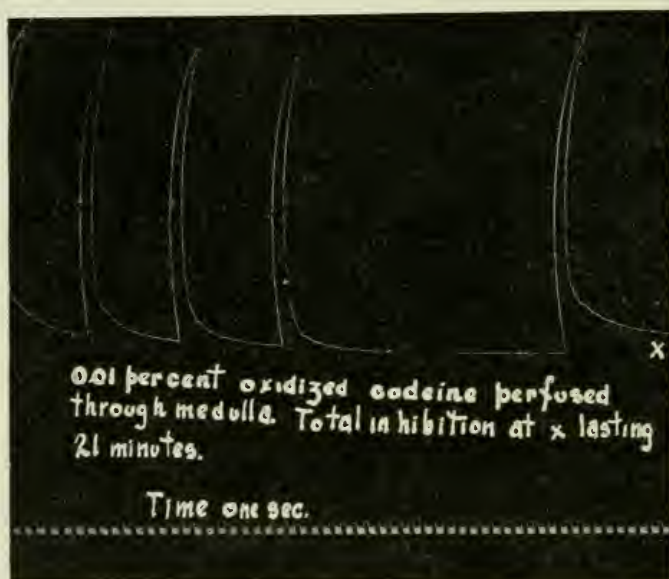


Fig. 4.—Inhibition produced by 1-20,000 oxidized codeine phosphate.

Codeine, however, does not stimulate the cardio-inhibitory center. The difference in action of these drugs may be due to the methoxy group which codeine contains, but which is absent in morphine. However, after oxidation with nitric acid, codeine exerts a more powerful and lasting stimulation than does morphine. In Experiment II, 5 c.c. of oxidized codeine produced total inhibition which lasted twenty-one minutes. It is apparent from this experiment that codeine is not oxidized in the body of the turtle as is morphine and hence it does not produce convulsions unless it is oxidized *in vitro* before it is perfused.

Apomorphine in fresh solution acts first as a powerful stimulant, but if its use is prolonged the medulla becomes paralyzed or at least irresponsive to known cardio-inhibitory stimulants. Upon standing, however, apomorphine loses this stimulating power since it effects no change in the heart rate when perfused through the medulla.

## SUMMARY

Morphine sulphate when perfused through the medulla of the terrapin (*Pseudomys troosti*) produces inhibition of the heart due to its products of oxidation, since morphine oxidized *in vitro* with nitric acid when perfused exerts a quicker and more powerful stimulating action than morphine *per se*.

Codeine exerts no influence on the medulla, due probably to the presence of a methoxy group. However, when oxidized it, too, exerts a powerful stimulating action.

Apomorphine first stimulates, then paralyzes the medulla. Solutions which have been exposed to the air and hence oxidized are ineffective.

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# CHEMICAL CHANGES OF THE BLOOD UNDER THE INFLUENCE OF DRUGS. II. MORPHINE\*

BY HARRY V. ATKINSON AND HAROLD N. ETS

## INTRODUCTION

THIS study of the chemical changes of the blood of dogs under the influence of morphine was undertaken because of the influence of morphine on the alkali reserve and oxidations in the body. Determinations of sugar, fat, cholesterol, and creatinine were also made on the same blood sample in order to determine the relation of these changes to those above. We are also interested in the possible formation of carbohydrate from fat which Higgins and Means<sup>1</sup> have suggested might take place during morphine narcosis.

## OXYGEN AND CARBON DIOXIDE

Filehne and Kionka<sup>2</sup> injected rabbits with one grain of morphine, and found a decrease in the carbon dioxide output and oxygen absorbed. They also noted an increase in the blood carbon dioxide and a decrease in the blood oxygen, and explained it as due to respiratory depression.

Loewy<sup>3</sup> measured the oxygen consumption of two men in normal sleep, and two in morphine sleep, and found a small drop in the oxygen consumed under morphine.

Boeck and Bauer<sup>4</sup> found a decreased carbon dioxide production and oxygen consumption in a dog under morphine, while in a cat in which morphine acts as an excitant, the gaseous exchange was greatly increased. A 50 per cent decrease in the metabolism of dogs and rabbits under the influence of fairly large doses of morphine was found by Fubini.<sup>5</sup>

Higgins and Means<sup>1</sup> in experiments on three men found either a slight decrease or no change in metabolism after giving one-fourth grain morphine, very little change in oxygen consumption, and a drop in the carbon dioxide elimination per minute which resulted in a drop in the respiratory quotient.

Chanutin and Lusk<sup>6</sup> determined the metabolism of two dogs under the influence of morphine. They gave their dogs subcutaneous injections of morphine in doses varying from 12 mgm. to 20 mgm. The first dog remained absolutely quiet in the calorimeter. Its metabolism decreased on an average of 6.2 per cent. The average respiratory quotient for this period was unchanged. A second dog manifested increased irritability with heightened reflexes after administering the drug. The basal metabolism of this dog increased by an average of 10 per cent. The average respiratory quotient increased during this period.

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## ALKALI RESERVE

The increase in the alkali reserve of the blood is indicated by the experiments of Barbour, Maurer, and von Glahn<sup>7</sup> who observed in fasting men an increase in the alveolar carbon dioxide under the influence of the injection of small amounts of morphine; and by the findings of Underhill, Goldschmidt, and Blatherwick<sup>8</sup> who have, in a similar manner succeeded in producing, in normal dogs, an alkaline urine which they found persisted for a period of 24 hours or more. They found also that after the urine had regained its usual acid character, a subsequent injection of morphine might fail to elicit an alkaline urine although the hydrogen-ion concentration might be diminished appreciably. On the day of the morphine introduction they also found an increased nitrogen elimination. Hjort and Taylor<sup>9</sup> found that morphine sulphate administered subcutaneously to normal dogs in doses of 10 mgm. per kilo of body weight caused an increase in the alkali reserve which is maintained at a high level for many hours. They also found that morphine injected in these doses into dogs treated with chlorine—so as to develop an acidosis—temporarily prolonged the maintenance of a high alkali reserve level, but that these doses exerted an unfavorable influence upon the ultimate outcome.

Gauss,<sup>10</sup> in studying the effect of morphine upon the alkali reserve of the blood of man and certain animals, found that morphine administered subcutaneously in the form of sulphate to rabbits, dogs, and sheep, distinctly increased the alkali reserve of the blood plasma of these animals. Likewise there was a slight but distinct rise in the  $p_H$  of the blood plasma. Excitement in dogs and rabbits decreases the alkali reserve of the blood plasma.

Gauss also found that the normal alkali reserve of the plasma of the lamb is distinctly lower than that of sheep, but morphine increases it in both animals. Repeated short interval injections of morphine do not tend to have a greater effect than a single injection. The alkali reserve increase as a result of the injection of morphine is greater in the rabbit than in either dogs or sheep. Gauss also states that in men who were given a therapeutic or slightly toxic dose (one-fourth to one-half grain) morphine did not have a marked effect when administered subcutaneously upon the  $p_H$  or the alkali reserve of the blood plasma.

## FAT

Bloor<sup>11</sup> found that morphine produced no change in the fat concentration of the blood of dogs during narcosis, but it did produce a considerable "after rise" during two or three days following.

## SUGAR

Lepine<sup>12</sup> states that Coze<sup>13</sup> was the first to observe the increase in the reducing power of the urine and the blood of animals under the influence of morphine. Eckhard<sup>14</sup> is given the credit for first finding a fermentable sugar in the urine under similar conditions, but in some cases he found that the urine was levorotatory, and this to be due to a conjugated form of glycuronic

acid—with morphine. This latter fact had been previously discovered by v. Mering,<sup>15</sup> Mayer,<sup>16</sup> and others. Morphine sometimes causes pentosuria.<sup>12</sup>

Luzzatto<sup>17</sup> found that morphine causes glycosuria in rabbits and this is confirmed by Araki.<sup>18</sup> However, Hirsch and Reinbach,<sup>19</sup> state that morphine is without effect on the blood sugar concentration of rabbits.

Ross<sup>20</sup> and also McGuigan<sup>21</sup> found that morphine almost doubles the concentration of blood sugar of dogs. Auer and Kleiner<sup>22</sup> found a maximum increase in blood sugar in normal dogs injected with morphine and about four times as great an increase in dogs with pancreatic deficiency.

Higgins and Means<sup>1</sup> found no appreciable increase in the blood sugar of man under the influence of morphine.

#### METHODS

Healthy and well-nourished dogs were selected and placed on a liberal mixed diet. The dogs were fed late in the afternoon and a normal sample of blood drawn the next morning from the left heart through a hypodermic needle. All the influences of digestion on the composition of the blood had ceased to operate by this time. The sample of blood was too small to cause any clapses due to hemorrhage. The dogs submitted without excitement in all cases. Immediately after drawing the first sample of blood, the dogs were given a hypodermic injection of one grain of morphine sulphate in divided doses. A second sample of blood was drawn two and one-half hours after the first and in some cases a third sample was taken seven hours after the first. In addition to the methods of analysis given elsewhere by the authors,<sup>23</sup> the oxygen capacity of the whole blood was determined according to the method of Van Slyke.<sup>24</sup>

#### EXPERIMENTAL DATA

The results obtained are given in Table I.

Upon inspecting Table I, it is seen that the carbon dioxide combining capacity of the blood plasma is increased from a normal value of 50 to 56 volume per cent, or a relative increase of 12 per cent two and one-half hours after the subcutaneous injection of morphine, and that this had further increased to 16 per cent above normal seven hours after the injection of morphine, but had returned to normal in 24 hours.

The oxygen capacity of the blood increased from 25.5 to 26.8 volume per cent, or a relative increase of 5 per cent in two and one-half hours and it had dropped 5 per cent below normal at the end of seven hours.

The normal blood sugar was found to be 0.09 per cent and this was increased to 0.14 per cent in two and one-half hours, or a relative increase of 53 per cent. This had dropped to 0.112 per cent in seven hours, but was still 23 per cent above normal.

There was only a very slight increase in the total blood fat in two and one-half hours, but a 9 per cent increase above normal existed after seven hours.

TABLE I

EFFECT OF THE SUBCUTANEOUS INJECTION OF ONE GRAM OF MORPHINE SULPHATE ON THE CHEMICAL COMPOSITION OF THE BLOOD OF THE DOG

DOG NO.	EXPERIMENT NO.	DATE 1921-22	CO <sub>2</sub>			O <sub>2</sub>			SUGAR			FAT			CHOLESTEROL			LECITHIN		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
			VOL. PER CENT	VOL. PER CENT	VOL. PER CENT	VOL. PER CENT	VOL. PER CENT	VOL. PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT
IV	1	Dec. 7	43	58	46	28.3	27.2	25.3	0.093			0.83	0.86		0.178	0.159		0.29	0.21	
IV	2	Dec. 8	46	56		28.0	40.3		0.100			0.74	0.83		0.173	0.231		0.27	0.27	
VI	3	Dec. 9	44	46		26.3						0.93			0.169			0.24		
VI	4	Dec. 12	52	64	64	22.2		19.8	0.086	0.097	0.097	0.87	0.91	0.91	0.112	0.130	0.104	0.30	0.18	0.34
VII	5	Dec. 15		54		20.3			0.068	0.120	0.097	0.85	0.95		0.125	0.139	0.104	0.47	0.45	
VII	6	Dec. 16	54	54		27.3			0.100	0.108		0.93	0.91		0.147	0.164		0.41	0.36	
VIII	7	Dec. 19	42	53	60	18.3	21.4	18.3	0.107	0.150	0.130	1.00	1.16	1.02	0.166	0.200	0.166	0.56	0.15	0.16
VIII	8	Dec. 20	53	63	60	16.8	27.3	27.3	0.098	0.125	0.103	0.89	0.93	1.10	0.156	0.104	0.187	0.50	0.39	0.41
VIII	9	Dec. 21	53	61	58	19.3		20.8		0.187		0.78	0.85	0.80	0.093	0.096	0.093	0.26		0.24
IX	10	Jan. 9	58	60	60	31.7	30.3	33.3	0.077	0.140	0.120	0.91	0.97	1.05			0.120			
IX	11	Jan. 11	55	55		30.8	34.8		0.072	0.118		1.00	1.00							
IX	12	Jan. 13	55	55		30.4	33.4		0.105	0.200		0.94	0.93							
IX	13	Jan. 16	47	50		30.4	30.2		0.970	0.143		0.95	1.05		0.167	0.131				
AVERAGES			50	56	58	25.5	26.8	24.1	0.091	0.139	0.112	0.88	0.94	0.98	0.15	0.15	0.13	0.32	0.29	0.29
CHANGE, PER CENT				+12.0	+16.0		+5.0	-5.0		+53.0	+23.0	+6.0	+10.0					-9.0	-9.0	-9.0

Column A contains the readings taken for a normal dog just before injecting morphine; Column B, two and one-half hours after injecting morphine; Column C, seven hours after injecting morphine.



The cholesterol first remained constant and then dropped 13 per cent below normal in seven hours.

The lecithin showed a decrease from the start and remained about constant at 9 per cent below normal.

Based on a limited number of results, not given in the table, the creatinine increased from 2.5 milligrams per 100 c.c. of whole blood to 3.2 milligrams or relatively 28 per cent in two and one-half hours, but had dropped from this high level to 2.9 milligrams in seven hours or 15 per cent above normal.

#### DISCUSSION

The fact that the carbon dioxide capacity of the blood plasma at the end of two and one-half hours was relatively increased 7 per cent more than the oxygen capacity, is of interest in connection with its effect on the oxidations taking place in the body. This is even more striking at the end of seven hours since by this time the carbon dioxide capacity had increased further and the oxygen capacity had dropped below normal, thus giving a relative difference of 21 per cent between these two. This would in all probability materially retard oxidations in the body.

In this connection the findings of Higgins and Means<sup>1</sup> are very significant. They found a fall from .75 to .70 in the respiratory quotients of human beings under the influence of morphine. These lowered respiratory quotients have been explained by these authors as being due to a change in the character of the metabolism, such as incomplete combustions, resulting in the formation of acetone bodies from fat or by changing of the fat to sugar. However, the formation of acetone bodies should cause the acidity of the blood, exclusive of carbon dioxide, to increase, which Higgins and Means<sup>1</sup> found not to be the case. This is confirmed in our experiments by the fact that the carbon dioxide capacity of the blood plasma is increased, indicating that there is no acidosis. The possible formation of sugar from fats would result in a very low respiratory quotient since an oxygen poor substance is being converted into an oxygen rich substance; this transformation has not yet been proved in dogs and human beings, but probably takes place in hibernating animals. Pembrey<sup>25</sup> also found a considerable fall in the respiratory quotients of dormice and marmots during their winter sleep. The respiratory quotients of his animals were much below that given by the oxidation of any one of the foodstuffs, fat, carbohydrate, or protein. In the experiments of Pembrey the body temperature of his animals dropped from 30° C. to 12° C., and this must of necessity diminish the oxidation processes taking place in his animals and would in all probability cause these to stop short of carbon dioxide formation, and this intermediate stage could be carbohydrate formation.

Chanutin and Lusk<sup>6</sup> found no decrease in the respiratory quotients of dogs under the influence of about three grains of morphine but they also record that their dogs vomited and defecated. We gave one grain of morphine sulphate in divided doses in order to prevent vomiting and rejected

all experiments in which there was vomiting because we found these results were very irregular. Usually there was no increase in the alkali reserve. We ascribed their results to vomiting and not to morphine. However, the changes in the composition of the blood under the influence of vomiting should be investigated more fully because of its effect on the alkali reserve of the blood which in turn may affect the metabolism.

The sugar increased 53 per cent during the first two and one-half hours, but was only 23 per cent above normal at the end of seven hours. Could this have been formed from fat? It is noted that the total blood fat showed very little change at the end of the period, but at the end of seven hours, it had increased 9 per cent. It is, of course, impossible to say whether the fat used to form glucose, if it be formed, would come from blood fat, in which case the blood fat should be decreased, or whether it would come from fatty depots of the body, or both. One would expect the maximum production of sugar from fat to occur at the time of the maximum change in the relation between the carbon dioxide capacity and the oxygen capacity.

It is noticeable that the cholesterols and lecithins decreased during the experiments.

#### SUMMARY

Morphine increases the carbon dioxide combining power of the plasma and the following constituents: Sugar, creatinine, and total fat. The lecithins, cholesterol, and oxygen combining power are decreased under the same conditions.

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## PATHOGENICITY OF ORGANISMS COMMONLY REGARDED AS SAPROPHYTES\*

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**A**MONG the bacteria usually classified as saprophytes are certain forms often associated with disease processes. These organisms are most frequently found in the body as harmless commensals and when isolated from inflammatory lesions the tendency is to regard them as contaminants and to make further search for known pathogenic bacteria. In many instances repeated examination reveals only the same organisms and one is forced to the conclusion that they are the etiologic factors concerned.

Even so indifferent an organism as the *B. subtilis* may, if accounts be true, present marked pathogenic characteristics. As long ago as 1897, Charin and deNittis<sup>1</sup> found that the hay bacillus attained considerable virulence by passage through animals. An extremely unusual case is that described by Lindberg<sup>2</sup> in which the *B. subtilis* was found as the cause of acute meningitis. Ophthalmia due to the same organism has been described by Silberschmidt,<sup>3</sup> and Spiegelberg<sup>4</sup> and others<sup>5, 6, 7</sup> have observed gastrointestinal disturbances apparently due to the hay bacillus.

In a case of meningitis secondary to otitis media in an infant, Greenthal<sup>8</sup> isolated the *B. acidi lactici* from the cerebrospinal fluid. Other saprophytic bacteria concerned in the causation of meningitis are *M. crassus*, *M. flavus* and *M. catarrhalis* as reported by Flexner<sup>9</sup> and more recently by Arce.<sup>10</sup> Schultz<sup>11</sup> describes in detail an instance of septicemia and acute vegetative endocarditis due to the *M. pharyngitidis siccae* which is usually saprophytic in the nasopharynx. This organism was isolated twice from the blood during life and at necropsy was obtained from the heart-valves and spleen. Teacher and Kennedy<sup>12</sup> cite a case of purulent meningitis due to the *M. flavus*.

This brief but incomplete review of the literature and our own experience indicate that saprophytic bacteria may not infrequently be responsible for severe and even fatal infection. In most laboratories it is the correct practice to view such organisms as contaminants or secondary invaders but their possible primary pathogenic characteristics must be borne in mind. In our own work we have occasionally obtained saprophytic bacteria in pure culture from such lesions as abscesses, meningitis, pleuritis, cholecystitis, etc. In a few instances the findings were so unusual as to warrant publication and so are recorded herewith.

### MICROCOCCUS CATARRHALIS MENINGITIS

This patient, a boy six months old, became acutely ill with fever and "apparent distress in the head." There was no nausea or vomiting, no

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pupillary changes. Physical examination was negative, but a lumbar puncture was performed and 15 cc. of slightly turbid fluid removed. The cell count showed 25 per cubic millimeter, globulin 3+ and smears, an increased number of cells, chiefly polynuclears and gram-negative, biscuit-shaped diplococci, extracellular and intracellular. Cultures yielded numerous colonies of similar cocci. These grew readily on ordinary media with the morphology and characteristic sugar reactions of *M. catarrhalis*. The child improved so rapidly that a second lumbar puncture was not performed. Ten months later the patient is reported as well.

#### STREPTOCOCCUS EQUINUS

The *S. equinus* frequently obtained in culture from the exposed surfaces of the body has been isolated by us from an infected hydrocele. The patient, a farmer sixty-four years of age, presented a large painful swelling in the



Fig. 1.—Cerebral abscess due to the *B. proteus vulgaris*.

right scrotal region of four years' duration. When removed, the testicle and epididymis appeared normal. The tunica vaginalis was greatly thickened and filled with thick creamy pus. Histologic examination of the tunica vaginalis shows the surface covered with a layer of granulation tissue markedly infiltrated with polynuclear, mononuclear leucocytes and eosinophiles. Newly formed blood vessels are present in large numbers. Many of the larger mononuclear cells resembling endothelial leucocytes are filled with old blood pigment. The surface of this granulation tissue is covered with a layer of fibrin and polynuclear leucocytes.

Smears and cultures from the pus yielded a streptococcus finally identified as the *S. equinus*. The organism grew readily on blood-agar, producing no change. In semisolid media there was fermentation of salicin but no fermentation of lactose or mannite.

In this connection it is of interest to refer to an instance<sup>13</sup> in which a



closely related organism, the *Streptococcus fecalis*, was isolated from the blood of a patient suffering from acute appendicitis.

#### BACILLUS FECALIS-ALKALIGENES

This organism, a normal inhabitant of the intestinal tract has frequently been suspected of causing profound infection. Now and then the *B. fecalis alkaligenes* is encountered as the cause of prolonged febrile conditions which in certain instances resemble typhoid fever.<sup>14</sup> In a few of these cases<sup>15</sup> the lesions of typhoid fever are present; in others no such pathologic changes are found.

In our case, the patient, a man forty-three years of age entered the hospital complaining of intestinal hemorrhage. No other historical facts were ascertained. On examination, jaundice was noted, the heart and lungs were found normal, spleen slightly enlarged and the liver distinctly enlarged. Tympanites present. Leucocyte count 9,000 and one Widal reaction reported positive. The Widal reaction was not repeated and no blood culture was taken.

The necropsy revealed acute suppurative peritonitis involving the right half of the peritoneal cavity, acute pyelitis, multiple abscesses of liver. The appendix showed no evidence of primary inflammation. No hyperplasia of the lymph follicles or Peyer's patches and no ulcerations were found. The histologic examination revealed an unusually large number of plasma and endothelial cells in the mucosa. The abscesses within the liver consisted of masses of polynuclear leucocytes in various stages of necrosis, a few large mononuclear cells and numerous endothelial leucocytes at the periphery. In the center of the abscesses were seen colonies of bacteria, chiefly short slender rods. No cocci identified. Smears and cultures from these abscesses yielded the *B. fecalis-alkaligenes* in large numbers. No other bacteria found. The peritoneal exudate, however, showed mixed infection, streptococci present.

#### BACILLUS AEROGENES

The *B. aerogenes*, also a normal inhabitant of the intestine, is frequently found in water and milk. Occasionally it occurs as a secondary invader in infections of the genitourinary tract, but is rarely encountered elsewhere. In the case herein cited, the *B. aerogenes* was isolated in pure culture from the pus derived from an acute cholecystitis.

The patient, a married woman, thirty-five years of age, with negative past and family history, developed jaundice, temperature 103° F., pulse 130, respirations 30 and pain and tumor mass in the upper right abdominal quadrant. The blood showed a leucocytosis of 16,000 and a polynucleosis of 80 per cent. At operation a greatly enlarged and inflamed gall bladder was found surrounded by many adhesions. The gall bladder contained "a pint of pus" which when received was streaked with blood and strongly mucoid in character.

Smears from the pus showed numerous pus cells and many gram-negative short bacilli surrounded by faint negative capsule zones. No true cap-

sules could be demonstrated, however. The pus when plated on blood-agar yielded colonies of one type. These were large, round, moist and mucoid, but did not have the strongly mucoid and confluent character of *B. mucosus*. It grew readily on ordinary media, giving the reactions of organisms classified as *B. aerogenes* and is not, we feel certain, the *B. mucosus*. Particularly noteworthy were the active acidification and coagulation of milk and gas formation. When injected into mice, guinea-pigs and rabbits, no marked pathologic changes were induced.

#### BACILLUS PROTEUS

The *B. proteus vulgaris* is well known as a cause of intestinal infection, being responsible at times for summer diarrhea although TenBroeck and Norbury<sup>16</sup> after extensive investigation arrived at the conclusion that it played a secondary part in infectious diarrhea. When found in lesions outside the intestinal tract, the *B. proteus* is usually associated with other organisms and is regarded as a secondary invader. In the case about to be related, it was isolated from a cerebral abscess and not associated with other bacteria.

The patient, a man aged twenty-eight years, with a previous history of chronic otitis media, entered the hospital in a partially comatose condition, with signs of cerebral disturbance. A diagnosis of abscess was made.

A radical mastoid and a decompression operation was performed on the left side and the brain exposed. The left temporal lobe was found to bulge considerably and when incised about one ounce of pus was obtained. The patient subsequently died and at the necropsy an abscess 2 x 4 cm. was found situated in the posterior portion of the left temporal lobe. Considerable clotted blood was present and replaced the pus previously removed. Cultures from the abscess and from the pus removed at operation yielded *B. proteus* in pure culture. No other organisms were found in smears from the abscess.

On histologic examination the abscess cavity is found lined by a thick layer of granulation tissue in which lymphocytes, plasma cells, and endothelial leucocytes are very abundant. A few newly formed capillaries are present and there is considerable interstitial hemorrhage. Within this layer are masses of polynuclear leucocytes and other cells in various stages of disintegration. The abscess is surrounded by a very distinct layer of fibrous tissue.

These few instances lead us to conclude that under favorable conditions many micro-organisms classified as saprophytes may induce in man profound lesions and even death. Bacteria classified as nonpathogenic when found in association with acute infectious processes are not necessarily secondary invaders or contaminants.

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## ACTINOMYCOSIS OF THE GASTROINTESTINAL TRACT: A STUDY OF FOURTEEN CASES\*

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THE diagnosis of actinomycosis of the intestinal tract is rarely made until late after sinus formation and extensive involvement of the intestines, liver, or other organs has taken place, and after surgical excision of the diseased area is impossible. Therefore, any mass which occurs in the lower part of the abdomen in the region of the ileocecal juncture, particularly one associated with a persistently discharging sinus, should be regarded with suspicion. Repeated examination of the discharge, pathologic examination of tissue from the mass or from the walls of the sinus, marked loss of weight and strength, pronounced anemia, flexion of the thigh without disease of the vertebral column or hip joint, and marked constipation rarely fail to establish the correct diagnosis.

### DIFFERENTIAL DIAGNOSIS

In the differentiation of tumors of the intestine and actinomycosis, carcinoma should be particularly considered. Its course is more chronic, it increases in size very slowly, anemia is not as pronounced, diarrhea with blood in the stools is more often observed, the mass is generally movable, is not tender, and shows little or no tendency to invade the abdominal wall or to form sinuses. Early diagnosis of both conditions can be made only by operation and pathologic examination of the tissue.

The differentiation of tuberculosis of the intestine and actinomycosis is also difficult until the later stages. Tuberculosis often forms a chronic, slow-growing tumor in the intestine, usually associated with pulmonary tuberculosis; diarrhea is common but there may be constipation. Sinuses do not form; the growth is slower, the mass generally movable, and tuberculosis bacilli can sometimes be found in the stools. The adjacent organs, particularly the liver, are not affected as in actinomycosis. Tuberculosis, carcinoma, and actinomycosis of the intestines all show a filling defect on roentgen ray examination of the colon. In all there is anemia, loss of weight and strength, and in tuberculosis and actinomycosis, elevation of temperature after secondary infection takes place.

Appendiceal abscess is often the beginning of actinomycosis infection,

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but most observers believe that the appendicitis is caused by the pyogenic bacteria and that this break in the mucosa allows the ray-fungi to penetrate the connective tissue in the wall of the intestine where they begin to grow. *Actinomyces bovis* has been found in the lumen of the appendix without inflammatory changes in the mucosa. Tubal abscess and localized peritonitis are more acute; they cause marked elevation of temperature. Pain is more intense, and constitutional reaction is more or less marked.

In gumma of the liver there is a history of infection; the Wassermann reaction is generally positive and other syphilitic lesions in the body establish the differentiation. In actinomycosis the abscess of the liver is always secondary to a primary focus in the intestine, or, rarely in the thorax. In amebic abscess of the liver there is a history of diarrhea and the ameba can often be found in the stools. There is no tumor in the lower abdomen and no discharging sinus. At operation the ameba can often be found in the purulent material in the abscess, while in actinomycosis the ray-fungi are easily recognized.

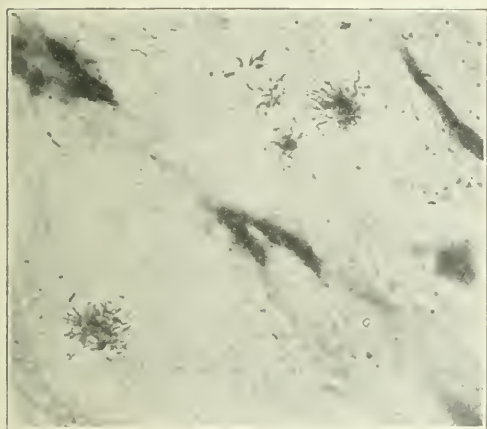


Fig. 1.

Fig. 1 (Case A210659).—*Actinomyces bovis* in the tissues (x 200).

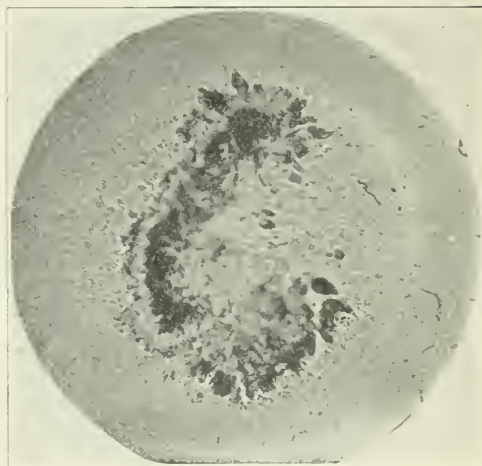


Fig. 2.

Fig. 2 (Case A210659).—*Actinomyces bovis* showing mycelia and clubs. Extensive round-cell infiltration and necrosis are around the organisms.

#### PROGNOSIS

The prognosis depends on the degree, nature, and extent of the lesions, the virulence of the organism, the resistance of the individual, and the time treatment is instituted. If an early diagnosis is made so that complete resection of the intestine and the diseased area can be accomplished, and vigorous iodid, and roentgen ray and radium treatments are given, the prognosis is fairly good. In the later stages, with involvement of the other organs in the abdomen and sinus formation, the possibility of a cure is always doubtful; most observers place the mortality from intestinal actinomycosis at 60 to 70 per cent.

In the series of fourteen patients studied in the Mayo Clinic, no report



of present condition could be obtained of three; one has been treated for only four weeks. Six of the remaining ten are dead, with an average length of life after onset of the disease of three years. One patient was operated on and had no symptoms of recurrence four years afterwards. One patient is apparently cured after four years of medical treatment, and two are markedly improved after taking iodides, and roentgen ray and radium treatments.

#### TREATMENT

The treatment may be surgical, medical, or hygienic. Surgical treatment is usually successful only when the disease is still localized and foci of infection can be completely removed. After sinus formation and secondary involvement of adjacent structures, the sinuses should be laid open, necrotic tissue curetted out, the wound packed with iodoform gauze or swabbed with strong tincture of iodine, and later irrigated with antiseptic solution, such as 1 per cent copper sulphate or 4 per cent formalin.

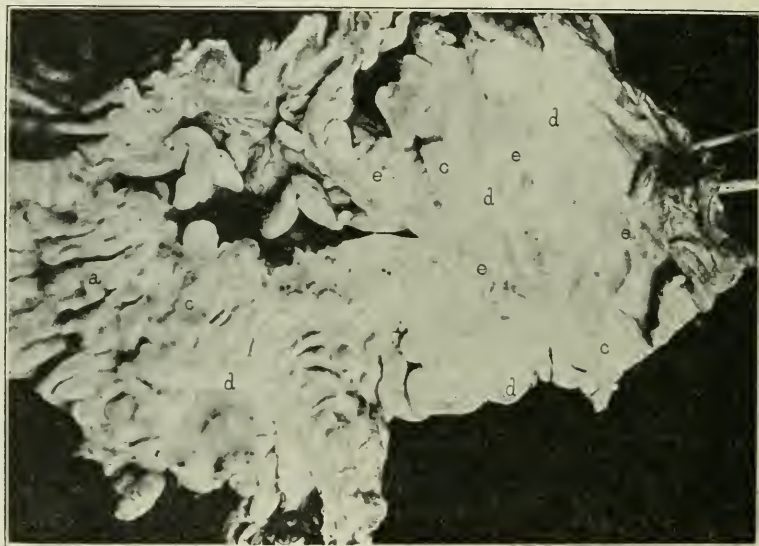


Fig. 3 (Case A210659).—Section of resected mass showing (a) cecum, (d) areas of marked fibrous tissue formation, and (e) areas of central necrosis.

The drugs generally used in the treatment are the iodides and copper sulphate. Potassium iodide is given by mouth in rapidly increasing doses of from 25 to 60 grains to 200 grains three times a day. Sodium iodide, 10 per cent solution, is given intravenously at first in doses of 20 to 30 c.c. and rapidly increasing to 100 c.c. a day. Copper sulphate, 0.5 grain three times a day is being used by some authorities, who report excellent results in both actinomyces and blastomycosis. Yeast by mouth daily, with salvarsan 0.2 to 0.6 gm. every six to eight days, has been tried, but without noticeably good results. Roentgen ray and radium, continued over a long period combined with the iodides, are giving the best results and are generally successful in the early stages of the disease. Tonics and stomachics are given.

Fresh air, sunshine, moderate exercise, an easily digested, nourishing

diet, or even forced feeding, are absolutely essential, especially in the later stages in which cachexia, and loss of weight and strength are marked.

However, a review of the literature<sup>1-13</sup> on the subject and study of this series shows that prognosis is unfavorable in all cases of actinomycosis of the intestinal tract. The mortality is 60 to 70 per cent. Treatment is successful only when diagnosis is early before sinus formation and involvement of adjacent organs, so that the primary foci can be entirely removed and later roentgen ray, radium, and the iodide treatments given. A study of fourteen cases of actinomycosis of the gastrointestinal tract in the Mayo Clinic since 1909 seems to warrant conclusions as follows:

1. Actinomycosis of the gastrointestinal tract is not so rare as has been generally believed; thirteen of the fourteen Mayo Clinic cases occurred in the last four years.

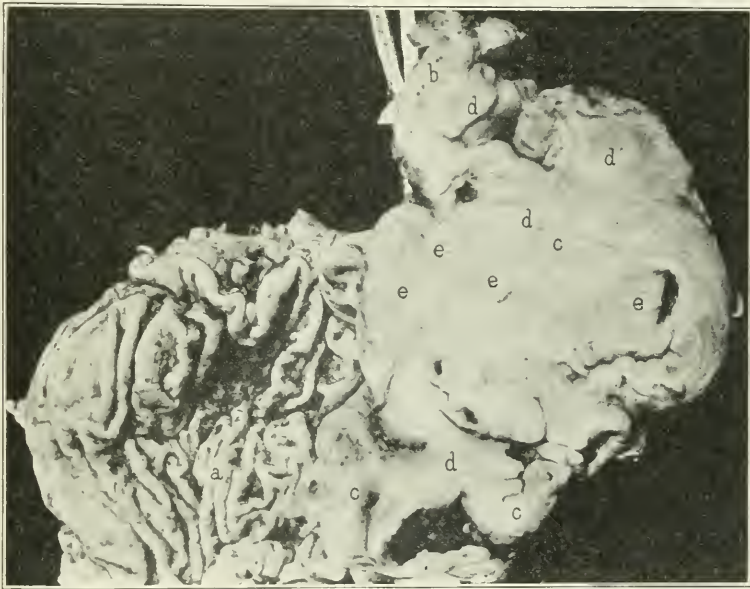


Fig. 4 (Case A278991).—Section of resected mass showing (a) cecum, (b) ileum, and (d) dense fibrous tissue, and (c) areas of necrosis.

2. The acute form is more common; it usually begins as a typical attack of acute appendicitis. There may be one severe or several mild attacks. Six of the patients in the series gave histories of operation when a ruptured, gangrenous appendix had been removed immediately preceding the appearance of the mass. The chronic form is insidious in onset with very slight indefinite pain in the lower abdomen. There may be practically no pain, the mass being the first sign of the disease.

3. In both forms marked loss of weight and strength, pronounced anemia, induration and a bluish red discoloration of the skin over the mass and around the sinuses, and pain down the right thigh with flexion of the thigh on the abdomen are characteristic symptoms. Diarrhea is not a constant symptom of the disease. Constipation was the rule in the fourteen cases.

4. The anaerobic organism, *Actinomyces Bovis*, which possibly normally

TABULATION OF CASES

CASE, DATE	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A30603 10/18/09	M, 51	Druggist, Iowa.	For five years attacks of dull pain in right side of abdomen lasting one to three days. July, 1908, severe pain for twelve hours and mass in upper abdomen, pain and tenderness increasing.	Tender mass in right upper quadrant of abdomen, increased liver myocytes found in dulness.	Intestinal actinomycosis with sinus formation, actinomycetes found in pus in January, 1916.	At operation, October 30, 1909, abscess in gall bladder region drained, 180 c.c. extension to liver pus and much necrotic tissue and thorax.  Second operation, December 12, 1909, pelvic abscess drained through right lower quadrant. Potassium iodide by mouth. January, 1916, another abscess drained in left lower quadrant.	Patient died, February, 1917, from extension to liver and thorax.
A148995 12/31/15	M, 40	Plumber, Ohio.	March, 1916, dull pain in left lower quadrant lasting five days, similar attack in July lasted three weeks, October 6, severe pain in right lower quadrant made operation necessary.	Large, hard, indurated mass in right lower quadrant with two sinuses discharging yellowish pus. Hemoglobin 41 per cent, leucocytes, 13,000.	Intestinal actinomycosis with sinus formation, pus showed actinomycetes.	Sodium iodide intravenously, and potassium iodide by mouth.	In 1917, the patient reported that no mass could be felt; he had gained in weight.
A207771 7/12/17	M, 35	Farm laborer, often worked with cattle with lumpy jaw, Montana.	For fifteen years recurrent attacks of dull pain in right lower quadrant. Since appendectomy, June, 1917, sinus in old wound drained yellowish pus.	Sinus draining yellow pus and hard, irregular mass in right lower quadrant.	Intestinal actinomycosis involving abdominal wall with sinus formation, actinomycetes from tissue from abdominal wall and pus.	Massive doses of potassium iodide by mouth and roentgen ray.	Patient died in 1919, cause unknown.

TABULATION OF CASES

CASE, DATE	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A207688 9/11/17	M, 33	Farmer, Saskatchewan.	Pain in lower abdomen since removal of ruptured gangrenous appendix in September, 1916. At laparotomy in November many adhesions and much scar tissue at site of previous operation was found. A persistently draining sinus made another operation necessary in March, 1917. Pain became more severe and in June abscess drained over right sacral region and in August abscess over left sacral region.	Distended abdomen, tenderness and induration of right lower abdominal wall with two sinuses to right and left of middle of sacrum discharging yellowish pus. Hemoglobin 40 per cent, leucocytes 14,800.	Abdominal actinomycosis with retroperitoneal abscess.	Four transfusions. Sodium iodide intravenously daily, 50 c.c. 10 per cent solution at first, gradually increased to 200 c.c., and tonics.	Patient continued treatment at home. No later report received.
A210659 10/12/17	F, 53	Housewife, husband a furniture dealer, Iowa.	In 1915, dull pain in right upper quadrant radiating to back with nausea and vomiting of bile. In June, acute generalized abdominal pain localizing in right lower quadrant associated with slightly increased temperature, anasarca, and constipation. Mass in right lower quadrant one month later.	Large, irregular, slightly movable, and slightly tender mass in right lower quadrant. Hemoglobin 58 per cent, leucocytes 12,000, roentgenogram of colon revealed filling defects in ileum and cecum.	Actinomycosis of ileum, cecum, appendix, and colon, actinomycetes in tissue (Figs. 1 and 2).	At operation, October 17, 1917, ileocecal coil, appendix, part of hepatic flexure, and 10 cm. ileum removed. (Fig. 3). Radium and roentgen ray, sodium iodide intravenously, and potassium iodide by mouth.	In 1919, patient reported condition good, no symptom of recurrence in abdomen.
A211906 10/25/17	F, 30	Housewife, husband electrical engineer, Iowa.	November, 1916, dull pain on both sides of lower abdomen with slightly increased temperature and metrorrhagia. At operation elsewhere in December, for suspected ectopic pregnancy, ovaries, tubes, and appendix found in suppurating mass. They were removed. Discharging sinus remained. July, 1917, large mass of cartilaginous consistency removed from pelvis.	Slightly tender mass in hepatic region, large, hard mass in pelvis. Slightly increased temperature, hematemesis, globin 20 per cent, leucocytes 14,000.	Intestinal actinomycosis with extension to pelvis and liver.	Four transfusions between December 4, 1917, and January 3, 1918. April 9, abscess below right breast drained. Frequent roentgen ray, large doses of potassium iodide by mouth, sodium iodide intravenously, tincture of digitalis, and Bland's pills.	Patient dismissed after six months to continue treatment at home. Death in 1919, exact cause unknown.



TABULATION OF CASES

DATE CASE,	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A224617 3/11/18	M, 26	Farmer, Saskatchewan.	In February, 1917, abdominal distention with chills, fever, and sweats. In April abscess in left upper abdomen discharged green pus. In May and July two more abscesses with persisting, discharging sinuses. Marked loss of weight.	Fluid in left chest and four discharging sinuses in lower and upper left quadrants of abdomen. Temperature 101, pulse 124, hemoglobin 50 per cent, leucocytes 15,000. Mass palpable below the umbilicus, skin over mass bluish red and indurated. Slight anemia.	Intestinal actinomycosis with secondary involvement of thorax.	Four aspirations of chest. Massive doses potassium iodide for one month. Treatment carried on at home.	Patient died in 1920.
A235668 6/19/18	F, 43	Housewife, husband farmer, North Dakota.	Dull pain in lower abdomen and mass in right lower quadrant for three months with dysmenorrhea and marked constipation.	Mass palpable below the umbilicus, skin over mass bluish red and indurated. Slight anemia.	Intestinal actinomycosis with involvement of abdominal wall.	Röntgen ray, and potassium iodide by mouth.	Several abscesses on abdominal wall ruptured, patient gained in weight, mass not increased in size.
A278991 7/15/19	M, 42	Farmer, Saskatchewan.	Soreness and dull pain across lower abdomen lasting one to three days, marked constipation. In June, 1919, severe attack of pain made operation necessary. Mass at ileocecal coil and many adhesions found, appendix removed.	Slightly tender, hard mass 8 cm. in diameter in right lower quadrant. Röntgenogram revealed filling defect of ileocecal juncture.	Malignancy of cecum.	At operation, July 25, 1919, large mass found involving ileocecal coil and adjacent parts of ileum, cecum, and ascending and transverse colon, 22.5 cm. resected. (Fig. 4). Six arsphenamine injections and massive doses potassium iodide by mouth.	Patient died February 19, 1920. Partial necropsy by home physician showed slight recurrence at site of operation.
A325922 7/21/20	M, 39	Farmer, Saskatchewan.	From 1913 to 1920, seven attacks severe pain in right lower quadrant lasting three to six days. At operation during last attack, ruptured gangrenous appendix and 10 cm. gangrenous bowel removed. Sinus drained since. Marked constipation and loss of 20 pounds.	Hard, indurated mass in right lower quadrant and three draining sinuses. Hemoglobin 50 per cent, leucocytes 13,800.	Intestinal actinomycosis with sinus formation, actinomycetes in pus from sinuses.	Röntgen ray, and massive doses of potassium iodide by mouth.	Patient died January, 1921, from cachexia and extension to thorax.

TABULATION OF CASES

CASE, DATE	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A332130 8/31/20	M, 36	Farmer, Iowa.	August, 1919, small lump in right lower abdomen; in March, 1920, it extended to umbilicus. Pathologic diagnosis from piece of excised tissue was chronic inflammatory tissue. Small abscess opened and drained, discharging sinus remained. Two attacks of diarrhea, usually constipation. Eleven injections of autogenous vaccine given without benefit.	Large, hard, irregular mass in right lower quadrant with draining sinus, skin bluish red and indurated. Roentgenogram revealed filling defect in cecum and ascending colon. Hemoglobin 55 per cent, leucocytes 9,800.	Actinomycosis of cecum and ileum involving abdominal wall.	Roentgen ray, massive doses potassium iodide, and tonics.	Patient not traced.
A336397 10/5/20	M, 27	Tile mill owner, Indiana.	May, 1920, loss of weight and strength associated with malaise and cough. In June, attack of pain in right lower quadrant with vomiting lasting one week, small lump in right lumbar region opened and drained, right thigh partially flexed on abdomen.	Large, irregular, hard mass in right lower quadrant with discharging sinus in right lumbar region. Leucocytes 12,000.	Intestinal actinomycosis with sinus formation, pus from sinus showed actinomyces.	Roentgen ray, and massive doses potassium iodide by mouth.	Patient not traced.
A341474 11/20/20	M, 30	Telegraph operator, Montana.	In 1918, attacks of dull pain across lower abdomen radiating to thigh and right hip, difficulty in extending right thigh. In 1917, severe attack in right lower abdomen, pain radiating to costal margin and right lumbar region. Unsuccessful exploration of gall bladder and right kidney elsewhere followed by profuse discharge pus for seven weeks, mild attacks of pain continued. Operation elsewhere disclosed gangrenous appendix and abscess of abdominal wall, sinus remained.	Four discharging sinuses and hard, tender mass in right lower quadrant, skin over mass bluish red and indurated.	Actinomycosis of cecum and abdominal wall, actinomyces found in pus from sinuses.	Roentgen ray, radium, and large doses potassium iodide by mouth. Treatment continued by home physician.	Condition much improved after two months.

TABULATION OF CASES

CASE, DATE	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A351274 3/1/21	F, 23	Housewife, husband general merchant.	December, 1918, general abdominal pain localizing in right lower quadrant. Pelvic abscess drained through vagina for three weeks. At exploratory laparotomy elsewhere, appendix, both tubes, and one ovary found in mass of adhesions and removed. January, 1920, recurrence of pain and hard, tender mass in right lower quadrant, second laparotomy. Since then dull pain in abdomen radiating down right hip and side, sinus at old incision drained since June, 1920.	Hard, nodular mass in right iliac fossa and pelvis not connected with uterus, two draining sinuses in lower abdomen, skin bluish red and indurated around sinuses. Moderate anemia, leucocytes 15,300.	Intestinal actinomycosis with extension to pelvis and abdominal wall.	Röntgen ray, potassium iodide by mouth, and sodium iodide intravenously.	Patient still under treatment.

inhabits the gastrointestinal tract, is the cause of the disease. The ileocecal coil is most often affected; the stomach and upper small intestine are practically immune.

5. Carious teeth, diseased tonsils, bad hygienic surroundings, overcrowding, or anything to lessen body resistance are predisposing factors. It is doubtful whether the disease is transmitted from cattle to man. In three of the fourteen cases there was a definite history of exposure to lumpy jaw in cattle.

6. A chronic discharging sinus in the lower abdomen, especially after operation and drainage of an appendiceal abscess, should arouse suspicion of actinomycosis, and repeated examinations for the ray-fungi should be made. A few cases are not diagnosed because the yellow granules are not found at the first examination. Repeated examinations of the purulent material over long periods, with pathologic examination of the tissue from the mass or from the walls of the sinuses, must be made before actinomycosis is ruled out.

7. Early operation and excision of the diseased area before the infection spreads to the surrounding tissues, frequent roentgen ray and radium treatments over the abdomen, and large doses of potassium iodid by mouth and sodium iodid intravenously offer the best hope of cure. In the later stages, after extension of the disease to the adjacent organs and sinus formation, radical surgical measures are of no avail. Some patients apparently are cured under treatment with radium, roentgen ray, and the iodides; others improve wonderfully only to have recurrence in from two to four years.

8. Death usually occurs from cachexia and extension of the disease to the liver and thorax. The average mortality is 60 to 70 per cent.

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## INTESTINAL ANTISEPSIS. EFFECT OF ANTISEPTICS ON A TYPE OF EXPERIMENTAL INTESTINAL TOXEMIA\*

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IT HAS been demonstrated<sup>1</sup> that the poisons, whose absorption is responsible for the toxemia following acute intestinal obstruction, are produced chiefly by the activity of the proteolytic group of intestinal bacteria. It is quite probable that various other disorders may have a similar origin and it is therefore of interest to determine the factors which may influence or control the growth of these organisms. The work of Kendall,<sup>2</sup> Hull and Rettger,<sup>3</sup> Torrey,<sup>4</sup> Cannon,<sup>5</sup> and others has shown conclusively that alterations in the diet may effect striking changes in the character of the intestinal flora. A high protein diet brings about an intestinal flora dominated by proteolytic bacteria whereas a carbohydrate diet, more particularly one containing definite amounts of either lactose or dextrine, suppresses the proteolytic group and brings about a predominance of fermentative or aciduric organisms. Experiments by the authors<sup>6</sup> have indicated that the toxemia incident to intestinal obstruction may be prevented or delayed according to the degree to which the offending proteolytic bacteria can be suppressed by dietary control. Unfortunately for the application of this method in therapeutics it was found that a stasis in the passage of the intestinal content ultimately led to the development of a proteolytic flora and resulting toxemia in spite of the carbohydrate diet.

One of the earliest methods used to check the growth of intestinal bacteria was the administration of the so-called intestinal antiseptics. Notwithstanding many conflicting and contradictory reports it is perhaps the general opinion among bacteriologists at the present time that the administration of these drugs in the methods in current use has little or no effect on the number of viable bacteria which may be found in the feces by cultural methods or the total amount of bacteria as determined by the method of Strasberger.<sup>7</sup> The treatment of various disorders by intestinal antiseptics administered in salol or keratin coated capsules, with the aid of the stomach or duodenal tube with lavage, colonic flushings, or appendicostomy with lavage using antiseptic solutions, is in common use. In general the results have been disappointing except in some specific infections where the chemical used has been specific for the etiologic organism; i.e., emetin in amebic dysentery. Such failures have contributed largely to the widespread opinion that intoxication arising through the activity of the intestinal bacteria plays little or no rôle in pathology.

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Death from acute intestinal obstruction is due to a toxemia. The toxic materials are produced in the intestinal tract by the action of the normal intestinal bacteria on the intestinal contents.<sup>1</sup> The symptoms can be reproduced in dogs by the production of closed isolated segments of various parts of the small intestine by surgical operations. When this is done a toxemia similar to that in acute obstruction occurs and the source of the toxemia is the closed segment since if this be removed the symptoms disappear. The proteolytic group of intestinal bacteria are the principal agents in the manufacture of the poisons absorbed from such closed loops of intestine since if these are rendered sterile by prolonged drainage into the abdominal cavity no toxemia can occur. The production of these closed segments of the intestine is a readily available method of inducing in the experimental animal a toxemia definitely due to the activity of the intestinal bacteria. Accordingly it affords an opportunity for testing the effect of intestinal antiseptics under conditions which can be definitely controlled and in cases where any effect would be easily manifest. In addition the method permits of the direct application of chemical antiseptics to the intestinal mucosa of such strength as would be impossible to secure by other methods.

EXPERIMENTAL PROCEDURE

Dogs were used for this work and the experiments were planned so as to give information relative to other problems of interest as well. This accounts for the large number of animals used. Isolated segments of the

TABLE I

ANTISEPTIC USED	NO. OF EXPTS.	STERILIZATION SECURED AS DETERMINED BY ABSENCE OF TOXEMIA OR NEGATIVE CULTURES
Ether	25	1 (Animal showed no toxemia and examination later revealed a perforated loop with no peritonitis).
Alcohol 70%	10	No segments sterilized.
Lysol 2%	8	No segments sterilized.
Silver Nitrate 5%	4	No segments sterilized.
Zinc Chloride sat. sol.	4	No segments sterilized.
Phenol 5%	5	No segments sterilized.
Salol sat. sol.	11	1 (Animal showed no toxemia and examination later revealed a perforated loop with no peritonitis).
Thymol 10 per cent in alcohol	4	No segments sterilized.
Camphor solid	4	No segments sterilized.
Menthol solid	4	1 (Animal showed no toxemia and examination later revealed a perforated loop with no peritonitis).
Naphthalein	4	No segments sterilized.
Dakin's Solution	3	No segments sterilized.
Beta Naphthol	2	No segments sterilized.
Thymol 10 per cent in alcohol	4	1 apparently sterilized.
Quinine Sulphate sat. sol.	5	No segments sterilized.
Mercuric Chloride	4	No segments sterilized.

upper jejunum were made by dividing the intestine in two places from eight to twelve inches apart and then reuniting the proximal and distal intestine around the isolated segment. The blood supply to the segment was not inter-

ferred with and care was taken not to injure the mesentery in any of the subsequent procedure. In most cases the intestinal segment was first thoroughly washed with water introduced at considerable tension and then with the antiseptic to be tested. The results are shown in Table I. In a majority of cases the toxemia incident to the production of the closed loop of intestine was not delayed by the application of the chemicals and in no case was sterilization secured.

Approximately 90 per cent of the animals died within a week following the operation and at autopsy there was found in a majority of cases a marked distention and perforation of the isolated loop with general peritonitis. In a few instances, however, the loops while markedly distended and discolored were not perforated and there were no evidences of peritoneal infection. The material in each of the loops was very toxic when injected intravenously in other animals and contained great numbers of bacteria. Ten per cent of the animals quickly recovered from the operation and never showed any subsequent toxic symptoms. Some of these were operated on again after several months and the loops examined. In most cases these were intact, slightly distended, and contained a thick, gray, foul-smelling, highly toxic fluid. This was found to contain great numbers of putrefactive or proteolytic organisms. The absence of toxemia in these cases has been shown to be due to the astringent action of the chemicals employed which checks the secretion of intestinal juices and consequent distention of the loop, and is not an indication of bactericidal action. Other astringents with no bactericidal properties such as tannic acid have a similar effect. In three experiments the loops were perforated, draining freely into the abdominal cavity, and there were no evidences of peritoneal infection. Cultures taken were negative. In these cases it was thought that a rapid distention and perforation of the loop must have occurred before there was opportunity for any marked bacterial proliferation. Doubtless the previous washings must have reduced the number of bacteria so that when perforation occurred, the bactericidal abdominal fluids could cope with the remainder and so secure sterilization.

#### DISCUSSION

The clinical use of antiseptics for their action on the intestinal bacteria is limited by a number of factors which play no part in these experiments. Chemicals must be selected which are little if at all absorbed from the intestine and so might cause systemic poisoning, which have no local irritant effect on the intestinal wall, and which still possess antiseptic properties when mixed with the intestinal content. It seems reasonable to suppose that if sterilization of the intestine by chemical means is a possibility it should have been secured in our experiments. Many of the usual antiseptics of a strength thought to be sufficient to sterilize a surgeon's hands were used without regard for their possible toxicity since they could be immediately removed. Furthermore they were applied directly to the segment of intestine in their full strength undiluted or mixed with the gastric or intestinal content. In spite of these most favorable conditions which could rarely if ever be se-

cured in clinical work, in over a hundred experiments it was not possible for us to secure a sterilization or to prevent this type of intestinal toxemia.

# CONCLUSIONS

1. The direct application of antiseptic solutions to short segments of the intestine in animals does not effect sterilization or inhibit the production of intestinal poisons.

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# LABORATORY METHODS

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## SOME POINTS IN METABOLISM USUALLY NEGLECTED BY THE PHYSICIAN\*

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BY J. F. MCCLENDON, PH.D., AND KENNETH MARCH, B.S.

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IN THE examination of the diabetic, since it is not possible to determine the quantity of sugar metabolized from the quantity found in the urine or the blood, the only recourse is the study of the complete metabolism. The calculations involved are complicated and hence not often resorted to by the clinician. The first point in this paper is to reduce the work of calculation to a minimum. It is necessary to determine the oxygen consumed, carbon dioxide eliminated, and urinary nitrogen secreted for a given period of time. Owing to the incomplete emptying of the bladder, very short periods may not be used except by catheterization. Folin has shown that the shortest period depending on voluntary emptying of the bladder is three hours. The capacity of portable metabolism-apparatus is usually not sufficient for more than about ten minutes' respiration. In order to obtain three hour values, more than one metabolism period during the three hours is necessary. After determining the urinary nitrogen (by Kjeldahl) calculate the protein-gaseous-metabolism, that is to say, the amount of oxygen and  $\text{CO}_2$  concerned in the burning of the protein. One gram of urinary nitrogen corresponds to 6.04 liters of oxygen and 4.88 liters of carbon dioxide. Estimate the total gas metabolism for the whole period of urine collection and subtract from it the protein-gas-metabolism. The result is the nonprotein-gas-metabolism. From the values obtained for carbon dioxide and oxygen, calculate the non-protein respiratory quotient by dividing the number of liters of carbon dioxide by the number of liters of oxygen. Find this value for the non-protein respiratory quotient in the first column of the following table. In the second column is given the number of grams of carbohydrate for each liter of nonprotein oxygen. Simply multiply the number of liters of non-protein oxygen by this figure and you obtain the number of grams of carbohydrate burned during this period. This value is independent of the origin of the carbohydrate whether from glycogen or from the carbohydrate of the food or from the body or food proteins. It is, therefore, the true index of the severity of diabetes. No diabetic is alive who cannot burn some carbohydrate but the less they can burn the more severe is the disease. The number of grams of fat may be obtained by multiplying the figure in the third column by the nonprotein oxygen in liters. This is true at any rate if

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the fat is totally burned. In case of ketonuria more complicated calculations must be made. The correctness of these calculations is reduced by ketonuria and by the storage of body fat but the storage of body fat rarely takes place in the diabetic and can be temporarily stopped in any person by going on a lower diet, and ketonuria may be reduced to a small value by diet in order to make the determinations, and hence the error will be small. We then have a value for the protein, carbohydrate and fat that is actually burned and if desired may calculate the calories of heat-metabolism by multiplying the number of grams of protein and carbohydrate by 4.1 and fat by 9.3. In fact the heat would be a little greater than this owing to the fact that these figures were obtained in experiments in which there was some loss of food in the feces.

## NON-PROTEIN METABOLISM

ONE LITER OF OXYGEN IS EQUIVALENT TO

NON-PROT.	GRAMS	
RESPIRATORY QUOTIENT	CARBOHYDRATE	FAT
.707	0.000	0.502
.71	0.016	0.497
.72	0.055	0.482
.73	0.094	0.465
.74	0.134	0.450
.75	0.173	0.433
.76	0.213	0.417
.77	0.254	0.400
.78	0.294	0.384
.79	0.334	0.368
.80	0.375	0.350
.81	0.415	0.334
.82	0.456	0.317
.83	0.498	0.301
.84	0.539	0.284
.85	0.580	0.267
.86	0.622	0.249
.87	0.666	0.232
.88	0.708	0.215
.89	0.741	0.197
.90	0.793	0.180
.91	0.836	0.162
.92	0.878	0.145
.93	0.922	0.127
.94	0.966	0.109
.95	1.010	0.091
.96	1.053	0.073
.97	1.098	0.055
.98	1.142	0.036
.99	1.185	0.018
1.00	1.232	0.000

If it is merely desired to determine  $\text{CO}_2$ , complicated apparatus is not necessary. The chief piece of apparatus is one for the collection of the gas sample. A spirometer of fifty liters' capacity is sometimes sufficient for ten minutes' respiration. Valves are used so that the expired air goes into the spirometer. It is very desirable to have the tube entering the spirometer pass through a condenser so as to reduce the temperature of the air to room temperature. For finer work some method of balancing the dome of the spirometer is

desirable. The simplest way to do this is to have a chain from the top of the dome pass up over the pulley wheel, and have a weight attached to the end of the chain equal to the weight of the dome in water, and one centimeter of the chain equal to the water displaced by one centimeter length of the wall of the dome. After you have the air sample in the spirometer, attach a glass tube on the mouth piece, insert this into a two thousand c.c. volumetric flask and by pressing on the dome force five liters of air through the flask and quickly remove the tube and insert the stopper; run in one-hundred c.c. of 0.1 normal barium hydroxide solution containing barium chloride from an automatic pipet and quickly insert the stopper, shaking hard until all of the carbon dioxide has been absorbed by the barium hydroxide; add a few drops of phenolphthalein and titrate back to colorless with 0.1 normal hydrochloric acid; subtract the number of c.c. of hydrochloric acid from one-hundred and multiply the result by 0.56 and the product will be the number of c.c. of  $\text{CO}_2$  per liter, of the spirometer air; multiply this figure by the number of liters of air collected in the spirometer and you will obtain the number of c.c. of  $\text{CO}_2$  exhaled during this metabolism period.

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### HEMATOMETRIC DIFFERENTIAL COUNTING\*

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THAT in the variations of the absolute number, and consequent numerical relationship of the different kinds of leucocytes found in the blood, in various diseases and pathologic processes, is to be found some specific point of diagnosis, as well as a true index to the body resistance, is something upon which a great amount of work has been spent by clinicians and hematologists of the past, in the effort to establish a definite and practical basis.

However, owing to the lack of a ready means of determining, upon a basis of accuracy, what the differential count really is under various conditions, little success so far has been attained in this direction. The differential count from stained films has long since proved a failure, in a practical way, in obtaining accurate and dependable data upon the subject.

(1) The size and construction of the different cells we have to deal with, vary in such a manner that it is impossible, by any ordinary means, to obtain a uniform distribution upon a slide or cover glass.

No matter how much care is exercised in spreading a drop of blood, or what method is employed, the tendency of the cells will invariably be to arrange themselves into three distinctly different formations, resulting to a greater or less extent, in the possibility of getting three distinct differential counts on the same slide.

The large lymphocytes will always be found in greater numbers along the edges of the slide, than in the middle; while at the beginning of the film,

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or at the end where the drop of blood was deposited, the small lymphocytes will be found proportionally high. Farther on toward the terminal end of slide, the polymorphonuclear cells and the eosinophiles will be found in greater numbers.

(2) Besides the difficulty encountered in this way, there is a process of agglutination of the cells of the blood, with their respective kind, which begins with the process of coagulation and interferes with uniformity of distribution. This is so marked in some cases that even in the short period of time it takes to transfer a drop to a slide and to spread it, there will be considerable grouping of lymphocytes and granular cells respectively.

This difficulty of obtaining uniformity of distribution, in making slide or cover glass smears, has been a matter of much concern among hematologists in work necessitating absolute accuracy of the differential count; so much so that mechanical devices have been perfected in the effort to overcome the difficulty.<sup>1</sup>

However, the difficulty of obtaining uniformity of distribution is not the only obstacle we have in the way of accuracy of the differential count by the slide or dry film method. Even if the uniformity of distribution desired could readily be obtained, the natural distance apart of such cells, normally present only in small numbers, is such that accuracy of enumeration cannot be expected upon a basis of anything less than an impractical number in routine work. A 2 per cent eosinophilia based upon a count of from 100 to 500 cells may in reality prove 0.5 per cent when from 3000 to 8000 cells are counted. Likewise, what appears to be 95 per cent polymorphonuclear cells, based upon the same small number counted, may really only mean 90 per cent or less when calculated upon a fair basis.

Recognizing the fact that the counting chamber is the only logical means of obtaining accuracy of the differential count, many attempts have been made by our earlier workers to perfect fluids that could be used, both as a diluting fluid and a differential stain at the same time. Turk in 1902<sup>2</sup> recommended that 2 to 3 parts of 1 per cent aqueous solution of gentian violet be added to a 1 per cent solution of acetic acid in order to get differential results. This solution, however, does not differentiate the eosinophiles from the neutrophiles and consequently does not answer the purpose in full.

Zappert in 1892<sup>2</sup> found that by filling the mixing pipette to mark 1 with blood and then with a 1 per cent solution of osmic acid up to about half the capacity of the mixing chamber, allowing this to act on the cells for a minute or so, and then filling the pipette to mark 100 with the following solution, the eosinophiles became deeply stained and could be readily enumerated among the other leucocytes.

Distilled water	55 c.c.
Glycerine	45 c.c.
1 per cent Aqueous Eosin Sol.	17 c.c.

The use of this fluid separately, in connection with that of Turk, enabled one to make a complete differential count on the counting chamber. How-



ever, the process is so complicated that apparently it never has gained favor with recent hematologists.

Following the work of Turk, Zollikofer, eight years later,<sup>4</sup> reported a differential fluid that was simpler and enabled one to make a differential count on the counting chamber in a single process. Two solutions are used of the following composition and mixed just before using in equal parts.

## A

Yellow Eosin, soluble in water	.05 gram
Formalin, concentrated solution	1 c.c.
Distilled water	100 c.c.

## B

Methylene blue "B X"	.05 gram
Formalin, concentrated solution	1 c.c.
Distilled water	100 c.c.

The writer, however, has not found these solutions satisfactory. The differentiation is not clear and the laking of the red cells incomplete.

Unof<sup>5</sup> recommends a mixture composed of equal parts of 5 per cent of formaldehyde, 1 per cent sodium chloride, a 5 per cent aqueous solution of eosin, "W G" and Grubler's polychrome methylene blue solution which is allowed to stain a half hour. This long process of staining in itself, however, would make the process impractical in busy routine work.

In 1919, the writer found that by modifying part A of Zollikofer's fluid by making it 0.5 per cent phenol and 0.5 per cent formalin instead of 1 per cent, this part alone became a perfect and satisfactory combined diluting fluid and differential stain. The formula complete may be set as follows:

Yellow eosin, soluble in water	0.5 gram
Formalin, concentrated	0.5 c.c.
Phenol 95 per cent	0.5 c.c.
Distilled water	100 c.c.

This fluid is staple and may be kept in large quantities in a stock bottle indefinitely. It causes a complete hemolysis of the red cells, swells the white cells to an extent considerably beyond their natural size and stains with the following points of differentiation.

*Polymorphonuclear Cells.*—These cells take on a brick-dust-red appearance of the granules with the nuclei remaining white and unstained. The tendency of the protoplasm of the cells is to shrink around the nuclei, leaving the cells shaped somewhat according to the number of nuclei present.

*Eosinophiles.*—Here the granules as well as the nucleus take on a bright red appearance, which may readily be distinguished from that of the neutrophils, even with the low power. The cells also remain spherical and stand out more prominently than the other granular cells.

*Lymphocytes.*—The lymphocytes, both small and large, remain hyalin and are distinguished simply according to their size.

The fluid gives a remarkably uniform distribution of the cells on the

counting chamber, apparently due to the action of formalin in preventing coagulation and agglutination to any degree, and enables one to get at once an accurate idea of what the differential count is in cmm. quantities.

*Differential Ruling.*—The writer has had a special differential counting chamber constructed, which, in addition to the regular Zappert-Ewing ruling, has a marginal ruling of 72  $\frac{1}{10}$  cubic millimeter fields, or a total ruling of 81 fields. A glance over these fields with the low power enables one to determine the absolute number of the various cells represented, upon a basis of from 3000 to 8000 cells in less time and with less trouble than it takes to make a count upon a basis of 100 cells from a dry film.

*Results.*—In the regular routine work, of both the laboratory and among hospital patients, the writer has found this method simple and dependable under all circumstances usually encountered. In contrast to the regular method of differential counting, it shows that the differential count is quite constantly a definite character in various infections and pathological processes and a greater classification of diseases and infections according to the character of the blood picture is possible. While presenting a little difficulty, in the beginning, to the technician accustomed to the dry film method, to the beginner, this difficulty is invariably found more easily mastered than the process of proper preparing and staining of blood films. It is only a matter of a little application to become accustomed to readily distinguish the various kinds of cells on the counting chamber, and, after this, accuracy of the count can always be depended upon.

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# THE QUANTITATIVE FLOCCULATION TEST FOR SYPHILIS. A COMPARISON OF 500 CASES WITH THE WASSERMANN, USING A SIMPLIFIED SACHS-GEORGI TECHNIC\*

BY HENRY M. FEINBLATT, M.D., BROOKLYN, N. Y.

THE realization of the nonspecific nature of the Wassermann reaction suggested to many investigators the possibility of devising a simpler test for the serum diagnosis of syphilis. As the belief grew that the Bordet-Gengou phenomenon is largely attributable to the presence of a specific globulin, many workers attempted to invent less involved methods to discover the existence of that substance in the serum.

## EARLIER WORK

The conception of precipitating the specific globulin was suggested to Sachs and Georgi by the researches of earlier workers. Michaelis had previously observed that syphilitic sera would sometimes form a precipitate with certain tissue extracts. Porges<sup>1</sup> in 1914 employed a precipitation method; but, when the menstruum was poor in sodium chloride, he obtained false positives. The latter results can be ascribed to the action of too weak a salt solution upon serum globulin.

Subsequently Meinicke used a two phase test. In the first step all of the globulin was precipitated by the addition of water. The second step consisted of the addition of a salt solution, which redissolved serum globulin but had no effect upon the luetic element. Parallel examinations with the Wassermann proved this method to be unreliable.

## THE WORK OF SACHS AND GEORGI

Working in Wassermann's laboratory, Sachs and Georgi,<sup>2</sup> in August of 1918, published the results of their researches along these lines. As this article was a war number, it was difficult to obtain, and it was only through the courtesy of the Surgeon General of the Army that the author was enabled to have access to it. Sachs and Georgi described their direct method, which, using only a cholesterinized antigen, produced a flocculant precipitate in syphilitic sera. No hemolytic system was required, the reaction being merely a globulin, antigen, amboceptor combination. Quantitative readings were made according to the degree of flocculation, which was accurately measured by means of the agglutinoscope.

Comparative tests with the Wassermann in 2770 sera yielded complete agreement in 2630 instances, or 94.94 per cent. In 2016 cases both were nega-

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tive; in 614, both were positive. In the 140 incompatible cases, the Sachs-Georgi was stronger or positive alone in 3.18 per cent; the Wassermann, in 1.88 per cent.

#### CONFIRMATORY REPORTS

Since that time numerous workers have investigated series parallel with the Wassermann. The results in the main indicate that there is better than a 90 per cent agreement between the two tests; that the Sachs-Georgi is more delicate and appears earlier after the primary sore; and finally that the Sachs-Georgi is a valuable adjunct to the Wassermann.

In October of 1918 Nathan<sup>3</sup> reported his findings in parallel tests with 704 specimens. There was accord in 92.8 per cent. The Wassermann was positive with a negative Sachs-Georgi in 29 cases; in 22 the Sachs-Georgi alone was positive. Nineteen patients from this last group gave a history of preceding syphilitic infection and one additional case was clinically congenitally luetic.

Raabe<sup>4</sup> found a 90 per cent exact agreement. Merzweiler<sup>5</sup> after making 700 comparative examinations concluded that the Sachs-Georgi is a complete substitute for the Wassermann. On the basis of his 1122 tests Messerschmidt<sup>6</sup> reached the same conclusion. Bok employed an improved quantitative technic and believed the flocculation method to be very reliable and more sensitive than the Wassermann. Baumgärtel<sup>7</sup> collected twenty-five thousand cases including seven thousand of his own and found agreement in over 90 per cent.

In this country, Hull and Faught<sup>8</sup> found accord in 88 per cent; Parker and Haigh,<sup>9</sup> in 93 per cent of their 520 cases. Levinson and Petersen<sup>10</sup> obtained parallel findings in 92 per cent of their 1042 tests. Of 62 of their cases in which the Sachs-Georgi was positive and the Wassermann negative, 57, or 92 per cent, gave evidence of lues either in the clinical history or the examination.

In 1921 Harryman<sup>11</sup> performed a parallel series upon 379 fluids from known syphilitic individuals. There was agreement in 91 per cent. In only 5 per cent of the fluids were the findings diametrically opposed. Of these 19 luetic patients, 16 were positive to the Sachs-Georgi alone and 3 to the Wassermann alone. He concluded that the flocculation test is the more sensitive and that it is positive earlier in neurosyphilis.

#### RELIABILITY OF THE WASSERMANN

It is well recognized that Wassermann reports from different laboratories are frequently contradictory. This is more often due to difference in technic than to error. Solomon<sup>12</sup> reported 3000 cases in which serum from the same specimen was independently examined by two standard laboratories. In this series, which he rightly considers a high water mark of agreement, there were 4 per cent of contrary reports. Considered on this basis, the accord between the Sachs-Georgi and the Wassermann is but slightly less than that which the latter itself gives, when separately performed by two different men.

Of the reliability of the Wassermann reaction where active syphilitic



lesions are present there can be no doubt. White, McWhirter, and Barber<sup>13</sup> report 100 per cent of positive Wassermanns in 177 cases of secondary syphilis, and 98.4 per cent with active tertiary sores. But, where we need it most, in latent lues, congenital syphilis, and neurosyphilis, the Wassermann most often fails us. Any test which can detect a definite percentage of Wassermann-negative cases of this group is entitled to recognition.

The frequency with which latent syphilis and a negative complement-fixation test coexist is suggested by the studies of Symmers, Darlington, and Bittman.<sup>14</sup> These investigators made careful postmortem examinations, both gross and histologic, of 331 cases which had had Wassermann examinations during life. Of those cases which showed definite anatomic evidence of lues, 31 per cent had, during life, been negative to one antigen and 56 per cent to the other.

Serologically negative tabes and meningovascular neurosyphilis are commonplace. The finding of a negative Wassermann with visceral syphilis is so ordinary that resort is often made to the therapeutic test in order to confirm the diagnosis. Consequently, the need is felt of a more delicate test, of one which will pick up certain of the cases which the Wassermann misses.

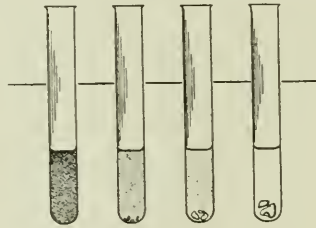


Fig. 1.

#### TECHNIC

The author's technic differs from the methods more commonly employed in two important respects; i.e., the preparation of the antigen and the use of centrifugalization.

Antigens differ greatly in their sensitiveness, depending upon factors which are not well understood. Individual variations in the tissue extracts selected are probably responsible for much of this lack of uniformity. It was found after repeated trials that supersaturation with cholesterol produces the most sensitive antigens.

After the addition of the antigen to the serum it is common practice to allow the mixture to stand for twenty-four or even forty-eight hours and then make the reading. Hull and Faught<sup>8</sup> first suggested centrifugalization. Gaeltgens in 1906 had used the centrifuge in the performance of the Gruber-Widal reaction, and it appeared logical to employ the same device with the Sachs-Georgi test. Comparative examinations showed that, in addition to being more rapid, the readings are more clean-cut and the flocculation, when present, is more pronounced.

*Preparation of the Antigen.*—Clean the fat from a fresh bullock's heart, grind the flesh to a fine consistency, and dry with filter paper. Pulverize

the meat with clean sand, simultaneously adding absolute alcohol in the ratio of ten c.c. to every gram of the heart tissue. Place this mixture in glass-stoppered bottles, shake, and incubate at 37° C. for ten days. During this time the mixture should be gently agitated twice daily. Next filter and place the filtrate in stock bottles, to be kept at room temperature.

*Cholesterinization of the Antigen.*—Add 0.6 gm. of cholesterin per 100 c.c. of antigen to insure supersaturation. Incubate this solution at 37° C. for three or four days, after which it will be ready for use. Prior to the performance of the test, it is preferable to reincubate the cholesterinized antigen at 37° C. for twenty-four hours.

*Performance of the Test.*—For use in the test the cholesterinized antigen should be diluted in the proportion of one part to nine of 0.9 per cent saline. It is essential to allow no considerable lapse of time to pass between the making of this dilution and the performance of the test. In fact, it is good practice to have the serum already in the tubes so that the antigen may be added immediately after the dilution is made.

Two tubes, each containing 0.3 c.c. of serum, are set up. Two different cholesterinized antigens are employed, one for each tube. To the serum add 1 c.c. of the 1 to 10 cholesterinized antigen, mix, and centrifugalize for fifteen minutes. Upon removing the tubes from the centrifuge, tap each tube gently with the finger and make the reading.

In negative reactions, the solution ascends in a turbid whirl, preserving the original cloudiness of the antigen; but there is no flocculation. In four plus reactions, there is a heavy, flocculant mass, the menstruum being clear. Three, two, and one plus results are represented by lesser degrees of flocculation.

#### PARALLELISM WITH THE WASSERMANN

Five hundred sera were independently examined by the Wassermann and the Sachs-Georgi methods. The Wassermann tests were performed by Dr. Archibald Murray and Miss Schultz, either in the Hoagland Laboratory or the Polhemus Memorial Clinic, while the author did the flocculation tests in the clinical laboratory of the hospital. The comparative quantitative results obtained in these cases are tabulated in Table I.

TABLE I  
PARALLEL OF 500 SACHS-GEORGI AND WASSERMANN TESTS

WASSERMANN		SACHS-GEORGI				
		neg.	one plus	two plus	three plus	four plus
	neg.	348	0	4	2	10
	one plus	5	3	4	2	3
	two plus	5	0	2	1	3
	three plus	0	0	0	6	2
	four plus	5	0	5	6	84

*Explanation of Table I.*—This chart is to be read like an annual baseball schedule. The figures placed in the vertical columns relate to the number of sera yielding results to the Sachs-Georgi indicated by the reading at the

head of the corresponding column. The figures placed in the horizontal columns relate to the number of sera yielding results to the Wassermann indicated by the reading at the extreme left of the corresponding column. For example, the figure 10 indicates the number of sera which were four plus to the Sachs-Georgi and negative to the Wassermann; the figure 1 indicates the number of sera which were three plus to the Sachs-Georgi and two plus to the Wassermann. The figures in italics relate to those sera in which identical quantitative results were obtained with both tests.

TABLE II

CLINICAL OPINION IN THE TEN CASES IN WHICH THE SACHS-GEORGI WAS FOUR PLUS AND THE WASSERMANN NEGATIVE

3 cases	congenital syphilis.
2 cases	treated syphilis.
2 cases	interstitial keratitis.
1 case	interstitial keratitis; mother and three sisters have four plus Wassermans.
1 case	spinal neurosyphilis; mother of luetic children.
1 case	mother of luetic children.

TABLE III

CLINICAL OPINION IN THE FIVE CASES IN WHICH THE WASSERMANN WAS FOUR PLUS AND THE SACHS-GEORGI NEGATIVE

2 cases	congenital syphilis.
2 cases	treated syphilis.
1 case	child with two luetic sisters.

TABLE IV

CLINICAL OPINION IN THE TWO CASES IN WHICH THE SACHS-GEORGI WAS THREE PLUS AND THE WASSERMANN NEGATIVE

1 case	treated syphilis.
1 case	no evidence of lues.

TABLE V

CLINICAL OPINION IN THE FOUR CASES IN WHICH THE SACHS-GEORGI WAS TWO PLUS AND THE WASSERMANN NEGATIVE

3 cases	treated syphilis.
1 case	congenital syphilis.

TABLE VI

CLINICAL OPINION IN THE FIVE CASES IN WHICH THE WASSERMANN WAS TWO PLUS AND THE SACHS-GEORGI NEGATIVE

3 cases	maternity, no evidence of lues.
2 cases	treated syphilis.

TABLE VII

CLINICAL OPINION IN THE FIVE CASES IN WHICH THE WASSERMANN WAS ONE PLUS AND THE SACHS-GEORGI NEGATIVE

4 cases	treated syphilis.
1 case	maternity, no evidence of lues.

An analysis of Table I shows an exact quantitative agreement in 443 sera, or 88.6 per cent. Altogether there were only 31 instances in which one test was positive and the other negative. Thus it may be fairly stated that the two methods were in accord in 93.8 per cent of cases. In 15 sera, or 3 per cent, one test was four plus and the other negative.

## ANALYSIS OF DISCREPANCIES

The clinical histories of all patients with contrary serologic findings were carefully examined principally with reference to the presence or absence of other evidences of syphilis. The diagnosis in each case represents the clinical opinion of the attending physician given before he was acquainted with the serologic findings.

TABLE VIII

SUMMARY OF ALL SIXTEEN CASES IN WHICH THE SACHS-GEORGI WAS POSITIVE AND THE WASSERMANN NEGATIVE

6 cases	treated syphilis.
4 cases	congenital syphilis.
3 cases	interstitial keratitis.
1 case	spinal neurosyphilis.
1 case	luetie family.
1 case	no evidence of syphilis.

TABLE IX

SUMMARY OF ALL FIFTEEN CASES IN WHICH THE WASSERMANN WAS POSITIVE AND THE SACHS-GEORGI NEGATIVE

8 cases	treated syphilis.
4 cases	maternity, no evidence of lues.
2 cases	congenital syphilis.
1 case	luetie family.

A consideration of the data presented in the above eight charts offers many points of interest. Fifteen patients with clinical evidence of syphilis, representing 3 per cent of the entire series, reacted positively to the Sachs-Georgi and negatively to the Wassermann. On the other hand, eleven patients with clinical evidence of syphilis, representing 2.2 per cent of the entire series, reacted positively to the Wassermann and negatively to the Sachs-Georgi.

There was only one Sachs-Georgi reading which could possibly be interpreted as a false positive. On the other hand, four maternity patients yielded Wassermann readings which might quite conceivably have been due to the presence in the circulation of placental products. Two of these four cases were followed up after delivery, and in both the Wassermann became negative without antisyphilitic treatment.<sup>15</sup>

Fourteen, or 45 per cent, of the discrepancies occurred in cases of treated syphilis. Here the serum is as apt to be negative to one test as to the other. Accordingly, it would appear advisable to employ both methods in a treated case before reaching a decision as to the need of further antiluetic therapy.

The data here presented would indicate that congenital syphilis, and



interstitial keratitis in particular, is more likely to be positive to the Sachs-Georgi than to the Wassermann.

Finally, it is evident that neither test is infallible and that either one may supplement the other in a definite percentage of cases.

#### CONCLUSIONS

1. Five hundred parallel Sachs-Georgi and Wassermann examinations showed an agreement of 93.8 per cent.

2. Of the 31 conflicting cases, 16 were positive to the Sachs-Georgi and 15 to the Wassermann.

3. Of the 16 patients who reacted positively to the Sachs-Georgi but negatively to the Wassermann, 15 presented definite clinical evidence of syphilis.

4. Of the 15 patients who reacted positively to the Wassermann but negatively to the Sachs-Georgi, 11 presented definite clinical evidence of syphilis.

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## EDITORIALS

### *Modifications of Gram's Stain*

IN 1884 Christian Gram observed rather by accident that in kidney sections stained with Ehrlich's anilin gentian violet and Lugol's solution and subsequently decolorized with alcohol, the stain was not removed from certain bacteria. The staining method devised by him at that time, has suffered little modification, and the classification of bacteria as gram-positive and gram-negative has since played an important rôle in bacteriologic studies. During the intervening thirty-eight years the only accepted modifications have been in the nature of rendering the gentian violet solution more stabile. Such an example is Sterling's modification. With a method which has thus stood the test of time, we might well consider variations or attempts at improvement, as entirely superfluous. The relatively rapid deterioration of the stain is however a distinct drawback, and even with the newer modifications the stain is by no means permanent. In many clinical laboratories and even in some bacteriologic laboratories in which the method is used

daily, the stain is allowed to age beyond the period of its reliability. We are inclined to use the same mixture until the bottle is empty rather than until the dye has lost its ability to stain satisfactorily.

It was partly for this reason that the Society of American Bacteriologists has offered standardized methods for the carrying out of the Gram stain. Variations even of these standardized methods have been suggested from time to time and are worthy of consideration and of acceptance if they are found to be of value. Atkins' and Burke's modifications have on the whole been received with approval. Kopeloff and Beerman suggest a further modification in which the advantages of Atkins' and Burke's methods are combined. The method consists briefly in (1) air drying and heat fixation, (2) staining with a mixture of thirty drops of 1 per cent aqueous methyl violet 6 B and eight drops of 5 per cent sodium bicarbonate, (3) fixation with iodine solution containing two grams of iodine, 10 c.c. of normal sodium hydroxide and 90 c.c. of water, (4) draining without blotting, (5) decolorization with 100 per cent acetone, (6) air drying, (7) counterstaining with 0.1 per cent aqueous basic fuchsin.

The authors state that the 1 per cent aqueous violet solution has relatively good keeping qualities and that the bicarbonate neutralizes acidity and improves the intensity of the stain in gram-positive organisms. If these two solutions are mixed, deterioration rapidly occurs, so that they should be kept separately. There is no danger of overstaining. Alkali in the iodine formula is believed to intensify the stain. Acetone apparently is a more satisfactory decolorizing agent than alcohol, and not only is it cheaper, but smaller amounts are necessary.

Kopeloff and Beerman claim for their method better keeping qualities, greater ease of execution and clearer differentiation than by the accepted Gram methods. Detritus which ordinarily takes the positive stain, is stained a pale pink by this method so that this obscuring feature is to some extent eliminated.

Scales has offered another staining method which while differentiating the same group of organisms as Gram's method, does not follow the theory of the method, since together with other differences, the iodine solution is not used. He uses as a stain cotton blue (Poirrier's Blue) made up in 5 per cent phenol solution. Only two solutions are used, the second being a combined decolorizing and counterstaining agent prepared by dissolving safranin in 95 per cent alcohol and adding an equal volume of acetone. The steps are described as (1) drying and fixation with slight heat, (2) staining with the first solution for twenty or thirty seconds, (3) washing, (4) decolorizing and counterstaining by application of the second solution, (5) washing and drying. Scales reports that in staining sixteen kinds of bacteria his method gave the same positive and negative results as were obtained with the aniline gentian violet stain.

His interpretation of the mechanism of the stain is that a physico-chemical reaction occurs. Phenol possesses a great affinity for living organic matter. The dye is soluble in carbolic acid. Thus the phenol serves

to fix the dye firmly within the cell substance. Decolorization and counterstaining appear to be purely physical phenomena in which osmosis through the cell membrane plays an important part. Thus acetone acts purely as a dehydrating agent and if dehydration is carried out rapidly water is completely removed from the cell and the dye remains fixed therein. This also occurs after rapid dehydration with filter paper. On the other hand, if the decolorizing and counterstaining solution contains 25 per cent or less of acetone the decolorization is more complete, showing according to Scales that the dehydration has not been sufficient to reduce the speed of osmosis outward through the cell membrane.

The utilization of acetone in the differential staining of bacteria appears to be a distinct advance. Modifications and improvements of the Gram stain are worthy of consideration and, if found reliable, of acceptance.

—W. T. V.

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### *Epidemiology and Bacteriology*

THERE seems to be, in the minds of some, confusion as to the relative scope and meaning of the words epidemiology and bacteriology. One man thinks that when he is teaching bacteriology he is instructing his students in epidemiology. This is often far from the truth. Epidemiology is far older and far more inclusive than bacteriology; in fact, compared with epidemiology, bacteriology is limited, and it is one of the handmaids of epidemiology. Two or three hundred years ago when epidemics were more frequent and often, though not always more destructive than at present, there was a word in common use among medical men and the intelligent laity. This word is loimology (loimologia), and was defined as the sum of human knowledge concerning pestilences or epidemics. This word will still be found in our dictionaries among those seldom used, although in common speech and in present-day writings it is never heard nor seen. The dictionaries of today contain another word, lemology, which is supposed to have the same meaning. This word also is obsolete and is not used in spoken or written language. The word epidemiology now bears the same meaning which three hundred years ago was attached to loimology. It means the sum total of human knowledge concerning diseases that prevail or may prevail among the people. Epidemiology, under present or past names, was well established, on at least some scientific basis, long before bacteriology came into existence. The epidemiology of Asiatic cholera was well understood long before Koch discovered the vibrio of this disease. In all human probability, Asiatic cholera has existed in certain parts of India, notably along the Lower Ganges, from time immemorial, but its history begins with the occupation of that country by the English. At that time travel became more extensive, more people



went out from the infected district, and those who did go traveled farther. It was soon recognized that Asiatic cholera is carried only by man, that it goes where the infected man goes, that it stops where he rests, and that there it may again be taken up by some other human being and carried farther. It was demonstrated that Asiatic cholera is not spread by the wind, or carried through the air, or carried by animals, but that its only carrier is man. As the means of transportation increased, Asiatic cholera traveled faster and farther. The first epidemic of which we have a history reached no farther than Asia Minor and northern Africa. Later epidemics took boat on the shores of Asia or from Egypt across the Mediterranean and planted themselves in Europe. Asiatic cholera has traveled by foot, by camel, by ox cart, by sail, by steam, and there is a possibility that it may yet travel by airplane. It was noticed in these epidemics that cholera did not appear in places which protected themselves by quarantine. In the early epidemics it was seen that local epidemics in these traveling pandemics were due to the contamination of drinking water. It was well recognized long before the discovery of the bacillus of this disease that the virus is discharged from the body in the fecal matter. After this discovery it required no great wisdom to recognize the fact that the spread of cholera can be limited by the destruction of the discharges of infected people and by preventing the contamination of drinking water-supplies; in short, the following facts concerning Asiatic cholera were well known and thoroughly recognized before this disease had any bacteriology: (1) It is a specific disease; (2) it never originates *de novo*; (3) its spread depends upon human intercourse between infected and uninfected areas; (4) the direction of its spread is determined by the paths of human travel; (5) places along these lines of travel may protect themselves by quarantine; (6) it is not transmitted through the air by the ordinary mingling of people; (7) it never appears on board ship except when persons infected at the time of sailing are on board; (8) it establishes itself and prolongs its stay in communities the drinking water of which becomes contaminated with fecal matter; (9) even in sparsely settled districts it is introduced and spread by the coming of infected individuals and in this way only; (10) on board ship it is spread from one to the other chiefly through infected water; (11) when a city water-supply becomes infected with the cholera vibrio there is a sudden outburst of the epidemic which soon reaches its maximum and then declines. The decline is due to the killing out of the cholera vibrio by the ordinary bacteria in the water; (12) the cholera virus grows and multiplies in the intestine of man and leads to necrosis of the epithelium; (13) drinking water in any part of the world infected with the cholera virus will cause the disease; (14) while cholera is for the most part distributed by drinking water, there is a possibility of its being distributed, though not widely, by contact, either direct or indirect; (15) cholera may be arrested by quarantine and disinfection. All these facts concerning the epidemiology of Asiatic cholera were known, were recognized, and determined the action of the first International Sanitary Congress held in Constantinople in 1866—long before

Koch discovered the vibrio of this disease; indeed, the discovery of the cholera bacillus has added only one thing to our control of epidemics of this disease. That one thing is an important thing, and it is the recognition of cholera carriers. What is true of Asiatic cholera is along the same line true of many other diseases. When it was ascertained in the fifties of the last century that the typhoid virus is discharged from the one ill with this disease by the bowels and through the urine, a large part of the epidemiology of this disease was explained. It is true that bacteriology has placed in our hands the ability to recognize typhoid carriers and to determine with certainty that transmission by contact may and does occur. When Villemin demonstrated in the sixties of the last century that the sputum and other excretions from tuberculous tissue carry the virus of this disease he became the scientific founder of the epidemiology of tuberculosis. Koch discovered the bacillus, but before him Villemin had demonstrated the contagious and infectious nature of the disease.

The epidemiology of a disease includes much more than its bacteriology. The epidemiologist needs to have some substantial knowledge concerning bacteriology, but the bacteriologist too frequently knows but little of epidemiology. Into the latter there must come, in addition to bacteriology, symptomatology, pathology, avenues of transmission, avenues of infection, habits and customs of the people, knowledge of housing conditions; in short, everything which influences the spread of disease. One would hardly expect to find an epidemic of trichinosis among Mohammedans or orthodox Jews, because neither of these eats pork. Typhus fever is a disease of housing and overcrowding. It has disappeared from certain countries when living conditions have been improved, and this may be *sine qua non* in the eradication of typhus fever. At the present time, with poverty and want so widely prevalent, the immediate eradication of typhus fever from Russia and Poland is nothing more than an idle dream. All claims that this disease has been stamped out by experts sent to countries for that purpose need to be closely investigated before they are accepted. A commission reaches some typhus infested community with the oncoming of hot weather and the disease abates. The coming of the commission and the oncoming of the hot weather fortunately occur simultaneously and the epidemiologist has no hesitancy in determining which of these has been the more important factor in the abatement of the disease. Typhus fever has been almost constantly imported into this country, some years largely, in others sparsely, for a long time and still it has not found a foothold or spread extensively because we are not, on the whole, a vermin-infected people, although we are in spots.

It is idle to talk about general rules of control as applied to all epidemic diseases. Epidemic diseases may be nutritional and wholly independent of any infective agent. They may be bacterial, protozoal, or parasitic. The virus may be air-borne, water-borne, insect-borne, borne by parasites, or disseminated by contact either direct or indirect. It is necessary to study the epidemiology of each disease both apart from and in conjunction with other diseases. Pneumonia as a sequel to measles or influenza is quite another dis-

ease than sporadic primary pneumonia. Much has been learned in the science of epidemiology by observations in armies. For more than one hundred years it has been quite plain to military students of this subject that the mobilization of an army is invariably accompanied by the development of one or more epidemic diseases. This is clearly understood when we recognize the fact that in mobilization a drag net is cast out over the country more or less extensively, as the case may be, and all kinds of infection are brought into camp. One man, possibly from an isolated farm where there was no danger or but little of his infecting others, is suddenly thrust into a great crowd of susceptible men while he bears, without evident harm to himself, a most virulent and deadly infection. French army surgeons recognized this in the epidemics of cerebrospinal meningitis which they studied in the early half of the nineteenth century. The mobilization records of every nation engaged in the late war show similar results. The accession of recruits has often proved more disastrous to an army than a battle. The mortality has been greater; the loss in morale has often been more widespread, and recovery has often been more difficult.

In the epidemics of typhoid fever in our own army during the Spanish War it was repeatedly shown that the incoming of recruits was followed by a recrudescence of the epidemic. Some of the regiments which assembled at Chickamauga during the Spanish-American War went to camp with only eight companies. After typhoid fever appeared in the Park each of these regiments received an accession of four companies. Within from ten to twenty days after the coming of the recruits the epidemic of typhoid fever took on new energy and the number of cases reported daily rapidly increased. Moreover, the new cases were not confined to the recruits, but the number of the old soldiers showing infection also increased. This condition or a similar one was repeated in the observation of the respiratory diseases in every cantonment in the United States during the World War. Typhoid fever and pneumonia epidemics increase not only with the incoming of recruits, but when soldiers from different cantonments mingle. This was so plainly in evidence during the World War that it happened more than once that a relatively small detachment from one cantonment to another changed the dominant type of the pneumonia—causing microorganism in the greater body. There are many points in these well-known observations which might be discussed with profit, but space forbids our doing so at this time.

—V. C. V.

### *Flocculation and Precipitation Reactions in the Diagnosis of Syphilis*

THE value of the Wassermann reaction in serologic diagnosis is unquestioned, but the method is not without its real disadvantages. The various ingredients entering into the complement-fixation reaction must be titrated with the utmost precision. Antigens are known to be variable. The German health authorities have attempted to eliminate this uncertainty by furnishing standardized antigen from a central laboratory. Antigen is, however,



but one of several variables. Complement tends to deteriorate with relative rapidity and since the interpretation of the test depends upon the disappearance of complement activity its deterioration may be mistaken for fixation. Again, serum is occasionally either wholly or partially anticomplementary. The former is readily detected with proper controls but in the latter case false positive readings may be made when the serum has absorbed some complement without the addition of antigen. Furthermore, many sera contain a natural hemolysin, the presence of which leads to erroneous negative conclusions. As Keim and Wile point out these and many other technical difficulties undoubtedly explain why, in spite of the fact that the Wassermann reaction has now been in use more than fifteen years, it is not, as yet, wholly dependable. Kolmer and his collaborators have in their extensive researches called attention to the many variables in the Wassermann reaction and have done much toward standardizing the technic.

The fact that alcoholic extracts of nonsyphilitic organs may be used in the test has discredited the specific antibody conception of the reaction. It is chiefly this fact that has inspired recent attempts to simplify the technic. The earlier interpretation of the Wassermann reaction as an antigen-antibody reaction has been questioned and it has been suggested that the phenomena observed are based on colloidal reactions. In 1907 several investigators suggested that a precipitate is formed in the positive Wassermann test and Jacobstahl showed with the ultramicroscope that such a precipitate actually is present when a mixture of Wassermann antigen and syphilitic serum is incubated for one and one-half hours. Attempts to produce a visible precipitation as indicative of syphilitic infection was first successfully carried out by Lange who developed the colloidal gold reaction. Of the ten or more precipitation or flocculation reactions that have been more recently described, the Meinicke, the Sachs-Georgi and the Kahn reactions have, because of their relative simplicity and because of their high correlation with the Wassermann test, received especial attention and study.

It has been shown that the globulins in syphilitic serums and spinal fluids are increased above the normal and the precipitation occurring in the various tests is supposed to be a reaction between seroglobulins and lipoid extract such that the resulting flocculate is a lipoglobulin aggregate. The Sachs-Georgi reaction is one of the simplest so far proposed. The technic consists in the incubation for from eighteen to twenty-four hours of a mixture of inactivated serum, sodium chlorid and cholesterinized heart extract. A positive reaction usually occurs at the end of that time although the tubes are customarily allowed to remain at room temperature overnight, when a second reading is taken. The test has been compared with the Wassermann reaction in over thirty-one thousand cases and in the aggregate there was an agreement of 86.9 per cent in all tests performed by various workers. Levinson states that the test may be elicited earlier and remains positive later than the Wassermann reaction and that it is not too rapidly influenced by treatment. Rice reports more rapid clearing up of the reaction in cases undergoing antisyphilitic treatment. In most of the work reported, the strongly



positive cases and the frankly negative have usually agreed when carried out by both methods. Sometimes, however, positive cases by one method have been questionable by the other, so that the differences have usually been ones of degree only. Rice found a certain number of cases in which there was a total disagreement. That these were in the minority is indicated by his conclusions that when roughly classified into positive and negative, the Sachs-Georgi tests agreed with the Wassermann tests in 99.44 per cent of one thousand cases tested. He found that while the test was less sensitive than the Wassermann in patients receiving treatment and in cases of cerebrospinal syphilis, it was equally as sensitive in untreated cases of primary, secondary and tertiary syphilis. He found in four cases in which the patient's serum was anticomplementary in the Wassermann test, that the Sachs-Georgi test gave a definite and reliable diagnosis in each case. This fact would of itself give the test considerable value in certain cases.

Keim and Wile point out that the main difficulty with both the Meinicke and Sachs-Georgi reactions lies in the fact that the end results are read after forty-eight hours' incubation, at which time the serums frequently show sufficient contamination to lead to false interpretations. The Kahn reaction is read after a much shorter interval and is even further simplified. In it the final precipitate is easily seen with the naked eye and in strongly positive serums spontaneous flocculation occurs, enabling interpretation as soon as the reagents are thoroughly mixed. Serums are mixed with small amounts of diluted cholesterinized antigen and with noncholesterinized antigen and the mixture is then vigorously shaken for three minutes. Sometimes at the end of this period a positive reading may be had, although as a rule readings are made after incubation overnight. The possibility of standardizing this type of reaction is increased by the fact that only one reagent is added to the serum. In primary syphilis there was an agreement between the Wassermann and the Kahn reactions in two-thirds of the cases while the remaining third showed a sensitiveness in favor of the Kahn reaction. Essentially the same findings were made in secondary syphilis. In tertiary lues, the Kahn reaction was found to be not as sensitive as the Wassermann reaction with ice box fixation but more sensitive than the Wassermann reaction performed with the short incubation period. Young in reporting a comparison between the Kahn and Wassermann reactions in five thousand and eighty serums, found an agreement of 93.03 per cent between the two reactions.

Serologic examination for the presence of syphilis has become so generally relied upon that any method which will increase the accuracy of the test should be utilized. Standardization of the original test is of the utmost importance. Other simpler reactions such as the Kahn and Sachs-Georgi tests may be used for confirmatory evidence. It may be that ultimately some simpler flocculation reactions will entirely supplant the Wassermann test. In the meantime, it must be borne in mind that the mechanism of none of the reactions is accurately known. We cannot explain why in syphilis and in practically no other disease some change has been produced in the

blood serum by which the precipitation reactions will become positive. We can explain the phenomenon in terms of colloidal chemistry and effect of proteins thereon, but at present, we cannot demonstrate why such reactions are found in few conditions other than syphilis. Reznikoff, in the November issue of this journal has shown that the colloidal gold reaction is a protein reaction, that the character of the curve varies with different proteins, that the curves are influenced by variations in the albumin-globulin ratio, and that in various clinical conditions such as diabetes, uremia, asthma, hypertension, etc., the amount of protein nitrogen and the percentages of albumin and globulin found in the blood serum vary greatly.

If the gold sol reaction, as a precipitation reaction, precipitates differently in different disease conditions, the possibility of similar variations in the newer flocculation reactions must be borne in mind. On the other hand, the future possibility of differentiation of several diseases by colloidal reactions, is conceivable.

In the meantime, the Kahn test and the Sachs-Georgi reaction appear to have a certain definite value as confirmatory tests when used with the Wassermann reaction.

—W. T. V.

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### *Sprue in Porto Rico*

ASHFORD<sup>1</sup> holds that sprue is due to a pathogenic yeast, *Monilia psilosis*. He believes, however, that this organism can cause the disease only among those living in a state of marked nutritional unbalance. He believes that the agricultural laborers of Porto Rico are living on such diets and he points out what he thinks to be the deficiencies in diet and what the clinical evidences of an unbalanced diet are. (1) A deficiency in fresh animal proteins and in fat-soluble A vitamin; (2) a probable deficiency in certain mineral constituents; (3) a bare sufficiency of B substance, the balance being easily disturbed when fresh vegetable constituents are reduced. These food deficiencies are indicated by the pallor, physical weakness and mental hebetude, together with the thousand and one functional aberrances which are included in the phrase "Effect of the Tropics." There is a very prevalent pigmentation of the skin, especially of the forehead, the jaws, and around the lips. The skin is atrophic and is especially susceptible to mycotic infection, particularly on the legs. Caries of the teeth, with pyorrhea, is highly prevalent. In children there is much nasal catarrh, bronchitis, and disturbances of breathing due to hypertrophied adenoids and tonsils. At all ages there is low blood pressure, with dyspnea, and a tendency to palpitation

<sup>1</sup>Am. Jour. Trop. Med., 1922, ii, 139.

and irregularity of the heart. People frequently complain of chilliness, and on this account they become addicted to the overuse of coffee. (There has never been any great amount of alcoholism in Porto Rico.) Myalgia, neuralgia, and indefinite pains are widely prevalent. Many of the people suffer from mental apathy, have difficulty in concentration of attention, and are defective in memory. In late adolescence there is frequent hystero-epilepsy and spastic conditions are observed in women who become chilled after toasting coffee or in men after unusual exertion. Among women scanty and irregular menstruation is common. Many people suffer from acid dyspepsias, with distention of the abdomen and constipation. There is chronic asthenia and speedy fatigue on effort is almost constantly in evidence. There is a wide prevalence of a waxy sallowness of the face, which deepens into actual and marked anemia, especially when there is infection with hookworm, malaria, or tuberculosis. On people in this condition *Monilia psilosis* finds suitable soil, grows and multiplies rapidly, and produces the clinical picture which we know as sprue. In a small number of cases sprue occurs in those in which food deficiency plays no evident part and in which the disease seems to come by sheer force of infection, but it is justifiable to suppose that some other factor has diminished the output of the digestive glands so as to permit the successful implantation of the organism.

—V. C. V.

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### *Change in Address of the Editor-in-Chief*

Until further notice the address of the Editor-in-Chief of this journal will be 535 North Dearborn Street, Chicago, Ill.

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## *ORIGINAL ARTICLES*

### NONTOXICITY AND ANTIPYRETIC EFFICIENCY OF TOLYSIN (ETHYL ESTER OF PARAMETHYLPHENYL CINCHONINIC ACID)\*

BY H. G. BARBOUR, M.D., AND E. LOZINSKY, M.D., MONTREAL, CANADA

AMONG the drugs most useful as antipyretics and antirheumatics, recent investigations have given prominence to the phenyleinchoninic acid group. Of these the acid itself and the Ethyl Ester of its paramethyl derivative are marketed under the Council-accepted name cinchophen (also under the proprietary name atophan) and Tolysin respectively. Emphasis was originally placed upon their capacity to increase the elimination of uric acid.

Hanzlik, Scott and others<sup>1</sup>, as well as Chace, Myers and Killian<sup>2</sup>, have however demonstrated their efficiency in rheumatic fever and allied conditions. Here the two drugs exhibit reduction of temperature and relief of pain, apparently in the same manner and degree as salicylates. It has, therefore, become of importance to determine more definitely than is at present known, the comparative toxic as well as antipyretic values of the phenyleinchoninic acid derivatives in relation to each other as well as to salicylates. In the accumulation of such data the relatively low toxicity of the ester became so apparent that attention was centered chiefly upon this drug.

The preparation of ethyl ester of paramethylphenyleinchoninic acid used in this work is known commercially as Tolysin. The drugs compared with it were cinchophen and aspirin.

The results on animals will be reported under the respective headings "Toxicity tests," "Antipyretic effects," and "Experiments accounting for the nontoxicity of Tolysin."

\*From the Department of Pharmacology, McGill University.  
Received for publication, Nov. 6, 1922.



## TOXICITY TESTS OF TOLYSIN, CINCHOPHEN, AND ASPIRIN

## MINIMUM LETHAL DOSE IN RATS

In these experiments acacia suspensions were used chiefly, each 100 c.c. containing 20 G. of drug. The injections were subcutaneous.

*Tolysin*.—The following doses exhibited no effect: 1, 2, 4, 10, and 10 G. per kilo (except that the 4 G. dose produced slight temporary lethargy). Twenty G. per kilo produced no effect on the day of injection, but the animal showed depression on the following day and died within 48 hours.

*Cinchophen*.—While 1 G. per kilo exhibited no effect, all larger doses were fatal. The following doses per kilo were given, and after each is mentioned the time within which death occurred: 2 G., 40 hours; 3 G., 36 hours; 4 G., 21 hours; 4 G., 40 hours; 8 G., 12 hours.

*Aspirin*.—One gram per kilo produced no result in one rat, but in another death occurred within 48 hours. The following fatal doses per kilo were also given: 2 G., 36 hours; 4 G., 20 hours; 8 G., 12 hours.

The rats were all old stock in excellent condition before injection. Absorption was rapid, if one may judge by the rate of reduction of the swelling.

*Tolysin per os*.—In four further experiments tolysin in doses per kilo of 2, 4, 10, and 10 G., respectively, yielded no effect, except for slight depression in one of the two receiving the largest dose.

From the above it appears that aspirin and cinchophen are at least ten times as toxic for rats as tolysin.

## MINIMUM LETHAL DOSE FOR DOGS

The discrepancy between the toxicity of the ester tolysin and the acid cinchophen, appeared so significant that more extensive studies upon both drugs were undertaken. So far as tolysin is concerned, apparently no literature exists on this subject. As regards cinchophen, Starkenstein<sup>3</sup> placed the m. l. d. for dogs at 1.5 G. per kilo; while in the case of aspirin, Block<sup>4</sup> found that two administrations of 0.7 G. per kilo each were required to kill. Chidichimo<sup>5</sup> places the m. l. d. at 0.5 G. per kilo. Neither of these investigators, however, was able to exclude vomiting.

In our first series of experiments hourly doses of the drugs were administered *per os*, in the form of aqueous acacia suspensions. The results may be summarized as follows (dosage per kilo being given):

*Tolysin*.—

1 x 5	G. =	5 G.	No effect.
12 x 1.67	G. =	20 G.	No effect.
7 x 4.29	G. =	30 G.	No effect.
6 x 5	G. =	30 G.	Slight incoördination and depression.
			Full recovery in twelve hours.
7 x 7.14	G. =	50 G.	No effect.

In the last cited experiment 275 grams (or over  $1\frac{1}{2}$  lb.) were given to a  $5\frac{1}{2}$  kilo dog without effect.

\*On two successive days, 6 doses each.

*Cinchophen.*—

3 x 0.33 G. = 1 G.	Depression; refused food. Recovered.
5 x 0.2 G. = 1 G.	Depression. Recovered in 12 hours.
5 x 0.25 G. = 1.25 G.	Marked depression; died within 12 hours.
5 x 0.33 G. = 1.5 G.	Marked depression. Died within 12 hours.

*Aspirin.*—In this series of aspirin experiments, vomiting occurred in every case, although in half of them the drug was administered in formaldehyde-hardened gelatin capsules, as indicated by the letters F.-G. Where vomiting occurred in these cases, the capsules were usually returned intact, so that the approximate proportion retained could be estimated.

The following doses were recovered from:

3 x 0.17 G. = 0.5 G.	(F.-G.); (part retained).
2 x 2.02 G. = 0.5 G.	(F.-G.); ( $\frac{1}{8}$ retained).
1 x 0.5 G. = 0.5 G.	(F.-G.); ( $\frac{1}{3}$ retained).
1 x 0.5 G. = 0.5 G.	(F.-G.); ( $\frac{1}{5}$ retained).
5 x 0.15 G. = 0.75 G.	( $\frac{1}{5}$ retained).
1 x 1 G. = 1 G.	(part retained).
6 x 0.17 G. = 1 G.	Marked depression; died within 12 hours.

The following fatalities occurred:

5 x 0.2 G. = 1 G.	(Total 2 G.) (part retained). (Same dog as last—second day). Died.
6 x 0.17 G. = 1 G.	(F.-G.) Depressed. Died within 40 hours. ( $\frac{1}{2}$ retained).
7 x 0.21 G. = 1.5 G.	(part retained). Marked depression. Died within 12 hours.

Owing to the amount of vomiting in each case, it was impossible to determine how much of the drug was retained. So that, from the above results one can only judge that 1 G., if absorbed, would be fatal. The experiment in which one gram was given and just half of the capsules retained, resulted in a slow (40 hours) fatality; whence it seems probable that one-half gram per kilo is the minimum lethal dose.

*Chronic poisoning.*—In the next series of experiments it was planned to compare the toxicity of the three drugs by giving smaller doses four times a day over a number of days. For this purpose the following doses per kilo were selected:

Tolysin	1 G.	—	$\frac{1}{4}$ t. i. d.
Cinchophen	0.2 G.	—	$\frac{1}{4}$ t. i. d.
Aspirin	0.1 G.	—	$\frac{1}{4}$ t. i. d.

Vomiting was absent in this series. The tolysin was continued over six days, giving a total dose of 24 grams per kilo, with absolutely no effect upon the general condition of the animal. The cinchophen dog showed some weakness at the end of the first day, gradually increasing until on the morning of the third day it was unable to stand. It was found dead on the morning of the fourth day—2 grams per kilo having been given in all. At autopsy a unilateral bronchopneumonia was found, which may have been a contributing factor.

The aspirin dog retained all of the drug; which rarely occurs with the dose given. The four doses sufficed to produce toward the end of each day definite signs of intoxication, including complete inability to stand and shallow rapid respirations. Each following morning he had apparently quite recovered.

The same phenomenon was repeated five days in succession.\* It will be noted that the quantity of aspirin given was ten times less than that of tolysin, which under the same conditions, proved inert.

*A 24-hour series.*—In view of the above results it was thought that had the aspirin administration been continued at similar intervals overnight it would have been possible to produce in the blood a sufficient concentration of salicyl (or salicyl plus aspirin) to give a fatal result. A series of administrations was, therefore, conducted over a period of twenty-four hours, with eight three-hour intervals—making nine doses in all. In each case the individual doses were the same as in the series reported immediately before.

Tolysin was given in this way to one dog, and no effect followed a total of 9 grams per kilo in the 24 hours. Two dogs received cinchophen, both becoming extremely weak toward the end of the experiment. One died within 48 hours, but the other recovered after depression lasting four days. Hence, the minimum lethal dose of cinchophen, distributed over 24 hours, lies close to 1.8 G. per kilo.

Two dogs received in the same way a total of 0.9 G. per kilo of aspirin: but some vomiting followed each dose and both dogs recovered.

This series emphasizes further the difficulty of determining the m. l. d. of aspirin for dogs, on account of poor retention. Tolysin, however, was tolerated perfectly under the conditions, in ten times the dosage of aspirin which was rejected.

#### SUMMARY OF TOXICITY EXPERIMENTS IN DOGS

*Tolysin* produces neither vomiting nor other effects upon the general condition, even with doses up to 50 grams per kilo. It appears impossible to kill a dog with tolysin administered *per os*.

*Cinchophen* produces vomiting occasionally with oral doses upwards of 0.5 gm. per kilo. Four doses of 0.2 G. per kilo in a single day produced marked depression. The minimum lethal dose administered within eight hours is approximately 1.25 grams per kilo.

*Aspirin* in oral doses of 0.1 gram per kilo usually, and 0.2 gram per kilo constantly, causes vomiting. The former dose, if retained, produces marked depression after it has been repeated three or four times. A surely lethal dose is 1 gram per kilo, and the m. l. d. is probably 0.5 gram per kilo.

#### ABSENCE OF ALBUMINURIA IN TOLYSIN-TREATED DOGS

In three different dogs, given repeated therapeutic doses of tolysin, the urine was tested for albumin. The animals received: (1) 0.5 gram per kilo daily for four days, omitted for five days, then repeated daily for another four days; (2) 1 gram per kilo four times daily for six days; (3) 1 gram per kilo every three hours for nine doses.

In each case, the urine collected over the entire period of administration was negative for albumin.

\*In an experiment of H. P. Foran the same dose given three times a day produced a temporary depression with convulsions at the end of the third day.

ANTIPYRETIC EFFECTS OF TOLYSIN, CINCHOPHEN, AND ASPIRIN

Since analgesic and antirheumatic effects cannot be satisfactorily determined in animals, the index of therapeutic efficiency of salicylates and drugs of the tolysin type must be their antipyretic action. In dogs this can be well shown by producing, with concentrated *B. coli* vaccine, a continuous fever, which usually persists for 48 hours.

*Normal Dogs.*—Like other antipyretic drugs, tolysin does not reduce the temperature of normal dogs. Although 50 grams per kilo fail to affect visibly the general behavior of a normal dog, slowing of the pulse may be demonstrated after one or two grams per kilo. This was the only significant effect seen in two dogs.

The first weighed 5.5 kilos, and exhibited, fasting, a normal pulse rate of 96. 5.5 grams tolysin *per os* was followed within one hour by a fall in pulse rate to 72. In a second dog, weighing 10 kilos, oral administration of 20 grams tolysin reduced the pulse rate within 1½ hours from 108 to 96 per minute.

As a result of the manipulation each dog exhibited a temporary increase in pulse and respiratory rates.

The first dog's temperature was unchanged, while that of the second showed a temporary increase of 0.7° C.

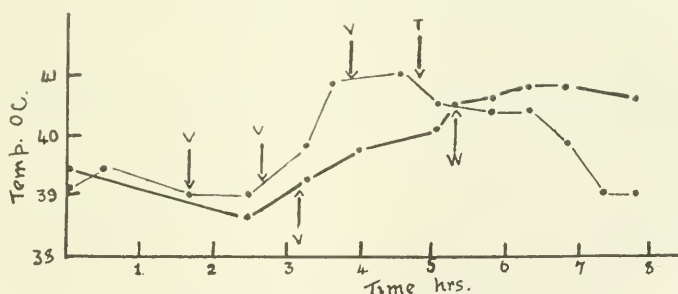


Fig. 1.—Antipyretic effects of tolysin in fever dog. Light line: Tolysin *per os*, 1.5 gm. per kilo, plus 250 c.c. water at T. Heavy line: Control Dog. 250 c.c. water only at W. V.—0.1 c.c. per kilo typhoid-paratyphoid vaccine (intravenous).

*Antipyretic Tests.*—As no *B. coli* vaccine was at first available, we began with intravenous injections of a mixed typhoid-paratyphoid vaccine.\* Injections of one or more tenths of a c.c. per kilo of this vaccine produced within 3 hours an increase of temperature amounting to from 1° to over 2° C. Unfortunately, even though the injection be repeated two or three times, the temperature usually falls without further interference almost as rapidly as it has risen; so that the determination of antipyretic efficiency becomes difficult.

To each of four dogs thus treated was administered *per os* 1 gram per kilo of tolysin, with the result that the temperature fell more rapidly than in any of the untreated animals. Two experiments with 1.5 grams per kilo yielded similar results; one of these is illustrated in Fig. 1, which shows also the curve of a control dog given 250 c.c. of water under the same conditions. The latter

\*Lederle's "Typhoid combined Prophylactic No. 2", containing per c.c. *B. Typhosus* 1,000 million, *B. Para "A"* 500 million and *B. Para "B"* 500 million.



exhibited no reduction in temperature, at least until the tolysin had reduced to normal the temperature of the other dog.

For more exact determination of antipyretic efficiency the *B. coli* vaccine (one million million killed bacilli per c.c.) was found preferable. In two preliminary experiments this vaccine was injected at 9:30 A. M.; five hours later 1 gram of tolysin per kilo was given *per os*. In the first dog the temperature, which had increased by  $1.2^{\circ}$  C., was reduced to normal by tolysin within 2 hrs.,



Fig. 2.—Antipyretic effects of tolysin in coli fever dogs; day after injection.

Light broken line: control (150 c.c.  $H_2O$ ). Light solid line: tolysin 0.75 gm. per kg. at arrow. Heavy solid line: tolysin 1 gm. per kg. at arrow. Heavy broken line: composite curve from two experiments; tolysin 0.3 gm. per kg. at arrow.

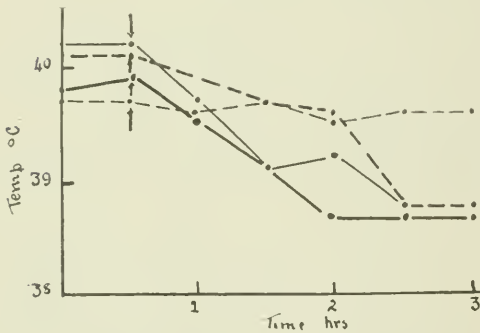


Fig. 3.—Antipyretic effects of tolysin, aspirin and cinchophen in coli fever dogs; day after injection.

Light broken line: control 150 c.c.  $H_2O$ . Light solid line: 0.15 gm. cinchophen per kg. at arrow. Heavy solid line: 0.1 gm. A. S. A. per kg. at arrow. Heavy broken line: 0.3 gm. tolysin per kg. at arrow.

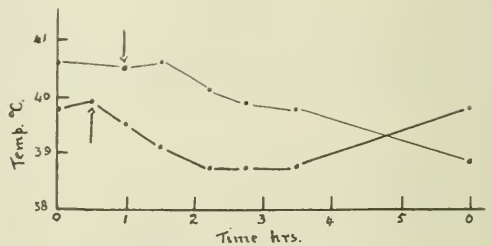


Fig. 4.—Persistence of tolysin effect.

Light line: 0.5 gm. tolysin per kg. at arrow. Heavy line: 0.1 gm. A. S. A. per kg. at arrow.

25 mins.; in the second animal recovery from a rise of  $1.3^{\circ}$  C. occurred within one-half hour of the injection. All of the subsequent experiments were performed upon the second fever day when the high temperature was found more stable.

To determine the minimal effective dose, gradually diminishing amounts were administered. We succeeded in reducing to normal the temperature of

two different *Coli* fever dogs by giving 0.3 gram per kilo. Smaller doses than this—e. g., 0.2 gram per kilo—were ineffective. For comparison, a number of fever dogs were treated with cinchophen and aspirin.

Antipyresis produced by doses of tolysin varying from 1 gm. down to 0.3 gm. per kilo is shown in Fig. 2. The maximum effect was obtained in from two to three hours after administration.

The respective effects of tolysin (0.3 gm. per kilo) cinchophen (0.15 gm. per kilo) and aspirin (0.1 gm. per kilo) may be compared in Fig. 3. It will be noted that the same degree of antipyresis was ultimately induced by all, but also that the two acids acted more promptly, although given in smaller doses.

Tolysin, the ester, is evidently more slowly absorbed. Compensating for this however is its more persistent effect, as seen in Fig. 4. Here is seen a prolonged antipyresis from 0.5 gm. per kilo tolysin, although 0.1 gm. per kilo aspirin acted more promptly.

While the minimal antipyretic dose of aspirin or cinchophen was found to be as low as 0.02 gm. per kilo of either, the effects produced thereby were submaximal and fleeting. The acute antipyretic experiment in the dog does not therefore afford the best criterion of comparative efficiency, owing to the differences in the rate of absorption. Where a more lasting effect is sought, as in acute rheumatic fever in man, the ratio of 15 gm. tolysin to 10 gm. aspirin or cinchophen appears to hold.

Even judging by the minimal antipyretic dose, in dogs tolysin presents a much more favorable  $\frac{\text{curative dose}}{\text{tolerated dose}}$  ratio than do the acids in question:

$$\left( \frac{C}{T} = \frac{\text{minimal antipyretic dose}}{\text{minimal lethal dose}} \right)$$

$$\text{Tolysin} \quad \frac{C}{T} = \frac{0.3}{>50} < \frac{1}{150}$$

$$\text{Aspirin} \quad \frac{C}{T} = \frac{0.02}{0.5} = \frac{1}{25}$$

$$\text{Cinchophen} \quad \frac{C}{T} = \frac{0.02}{1.25} = \frac{1}{63}$$

So that, even were 50 gm. per kilo fatal and the antipyretic effect of 0.3 gm. per kilo a fleeting one, tolysin would still have six times the "therapeutic range" of aspirin.

#### EXPERIMENTS ACCOUNTING FOR THE NONTOXICITY OF TOLYSIN

The finding of such a very large therapeutic range of dosage for tolysin led us to the conclusion that the rate of absorption must become considerably retarded as the dose is increased. This was put to the test by determining the amount of tolysin that could be recovered from the feces of dogs after various doses had been administered. This was done in three dogs given respectively: 0.5 gm. per kilo tolysin in one dose; 1.5 gm. per kilo in 7 hours; and 9 gm. per kilo in 24 hours. Additional tests were made in a case of acute

TABLE I  
ABSORPTION OF TOLYSIN

EXPERIMENT	TOLYSIN		CONDITIONS	DETERMINATIONS	TOLYSIN POSSIBLE IN SAMPLE	PARAMETHYL CINCHOPHEN RECOVERED	TOLYSIN EQUIVALENT	ABSORBED (Calculated)	
	Gm.	Gm. per kilo						Gm. p. kilo	Per Cent
Control A	1.5	-	mixed with 12.5 G. dog feces	*	0.50	0.40	0.44	-	-
Control B	12	-	mixed with 12 G. dog feces	2	5.00	4.63	5.09	-	-
Man (Ac. Rheum. Fever)	24	0.3	per os in 48 hours	2	1.97	0.00	0.00	0.3	100
Dog No. 3A	2.75	0.5	per os in one dose	2	0.65	0.00	0.00	0.5	100
Dog No. 41A	6	1.5	per os in 7 hours	12	1.72	1.04	1.15	0.5	33
Dog No. 44	45	9	per os in 24 hours	2	5.69	3.60	3.97	2.7	30

\*The results are based on averages from the given number of determinations.

Note:—A control "after-period" followed each absorption experiment except Dog No. 44, in which collection was continued over four days. No paramethylcinchophen was recovered in the after-periods.

rheumatic fever in man, where 0.3 gm. per kilo tolysin was given in 48 hours. Two control experiments were performed *in vitro*. In the first 1.5 grams tolysin was thoroughly mixed with 13.5 gm. dried dog feces; in the second 12 gm. tolysin was mixed with an equal amount of dried dog feces.

*Method.*—The following procedure (for the details of which we are indebted to a personal communication from Dr. M. S. Fine) was employed:

A 5 or 10 gram sample of dried feces was extracted with ether in a Soxhlet apparatus. This extract was saponified, using for each gram of extract 1½ grams KOH and 25 c.c. alcohol. Digestion was continued for about two hours on a water-bath, under reflux. After filtration, the filter paper was thoroughly washed with boiling alcohol and the alcoholic solution of soap was concentrated to a syrup; a minimum of distilled water was added and the solution boiled until all alcohol was removed.

The soap was then decomposed with dilute HCl, using methyl orange as an indicator. The fatty acid and paracinehophen were filtered off and dried together with the filter paper in a drying oven. The fatty acids melted and were absorbed by the paper, it being then possible to transfer quantitatively the paracinehophen to a weighed Gooch crucible. The paracinehophen was further purified by washing with benzol until free from fatty acids.

*Control Experiments.*—As will be seen from Table I, the tolysin was recovered quantitatively from the dried feces in a sample where 5 grams were present. In the smaller sample of the 1:9 mixture only 88 per cent of a possible half-gram were recovered. The error, therefore, may be as great as 12 per cent when such small quantities are dealt with. This will be found of no significance in drawing the chief conclusions from the following results:

*Absorption Experiments.*—The data from the case of acute rheumatic fever and the three dog experiments confirm the conclusions drawn from the pharmacologic evidence; for doses giving the maximum therapeutic effect, e. g., 0.3 and 0.5 gm. per kilo, respectively in man and dog, were apparently completely absorbed, no portion being recoverable in the feces.

But when the smallest dog dose was tripled there was no increase in absorption, the absolute amount being only 0.5 out of a possible 1.5 gm. per kilo.

Even where 9 gm. per kilo were given only 2.7 gm. per kilo were absorbed, and this amount would undoubtedly have been much less if the administration had not been extended evenly in 9 doses over 24 hours.

It is only necessary to add that where very large doses are given it is possible to observe clumps of tolysin powder in the feces of dogs.

We find, therefore, in tolysin a drug exhibiting the coincidence that the maximum limit of absorption is practically identical with the full therapeutic dose—apparently a new and fortunate peculiarity in pharmacologic behavior.

#### CONCLUSIONS

1. Ethyl ester of paramethylphenyleinchoninic acid (tolysin), administered *per os*, even up to doses of 50 gm. per kilo, or 5 per cent of the animal's own weight, produces no effect upon the general condition of dogs. Aspirin, however, may prove fatal in the oral dose of 0.5 gm. per kilo; 0.4 gm. per kilo,



given over a period of 9 hours, produces marked depression. The m. l. d. of cinchophen *per os* in dogs is about 1.25 gm. per kilo.

2. The respective minimal antipyretic dose over minimal lethal dose ratios for dogs are approximately:

$$\text{Tolysin} < \frac{1}{150} ; \quad \text{Aspirin} \quad \frac{1}{25} ; \quad \text{Cinchophen} \quad \frac{1}{63} .$$

Tolysin therefore seems to be the least toxic of all substances of demonstrated antirheumatic efficiency.

3. Tolysin exhibits a peculiarity in pharmacologic behavior in that the maximum limit of absorption from the intestine coincides essentially with full therapeutic doses. As emulsion is evidently absent, indefinitely large amounts are nontoxic, at least for dogs.

The authors take pleasure in acknowledging the assistance of Mr. Edward Tolstoi of the former Department of Pharmacology of Yale University, who performed a number of the preliminary experiments.

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## THE EXCRETION OF SUGAR IN THE URINE IN HEALTH AND DISEASE\*

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THE collection of accurate information concerning normal urinary sugar excretion was first made possible by Benedict and Osterberg,<sup>1</sup> who described in 1918 a method of estimating the sugar of normal urine. With this method they<sup>2</sup> found in two normal men an average daily excretion of 0.8 and 0.5 gram reducing substance (sugar) on a low carbohydrate diet, 1.0 and 0.65 gram on a medium carbohydrate diet, and 1.5 and 1.1 grams on a carbohydrate-rich diet. They also demonstrated that in normal individuals there is an increased sugar output in hourly urine specimens taken after meals. Kast, Wardell and Myers,<sup>3</sup> using the same method, demonstrated this "glycuresis" after meals in normal individuals and in diabetic patients. They showed that while the urine of a diabetic patient may be "sugar-free" in the morning, or several hours after a meal, large amounts of sugar may be excreted in the urine voided soon after the eating of food.

For this reason it has been emphasized by the latter workers that the use of a qualitative copper test on a morning urine specimen might be quite misleading in studying the carbohydrate tolerance of a patient. On the other hand, the determination of the amount of reducing sugar excreted in twenty-four hours in a series of cases would appear to be of value both in providing more information on normal sugar excretion and in showing the possible influence of disease on such excretion. Data are given in the present paper covering these points.

The original method of Benedict and Osterberg<sup>1</sup> for the determination of sugar in normal urine has the disadvantage of requiring the preliminary removal of interfering nitrogenous substances from the urine by precipitation with mercuric nitrate and sodium bicarbonate. That this is a laborious and time-consuming procedure is of some consequence in a study requiring many determinations of sugar. More recently Benedict and Osterberg<sup>4</sup> have described a new method for the determination of reducing sugar in normal urine which does not require this preliminary treatment with mercuric nitrate. The method is based on the reducing action of the sugar on a carefully controlled amount of picric acid and sodium hydroxide, following the treatment of the urine with specially purified bone black; the interfering action of creatine and creatinine is prevented by the addition of acetone, which dissipates the color due to these two compounds, without greatly in-

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fluencing the color arising from the sugar. In order to obtain accurate results, it is essential that the amount of sugar in the urine used for the test should be between 0.5 and 1.5 mg., i.e., not far from the amount (1 mg.) present in the standard. Benedict and Osterberg's results found by this method agree very closely with those obtained by their former procedure.

We have compared these two methods and also those of Shaffer and Hartmann<sup>5</sup> and Myers<sup>6</sup> (our observations were completed before the appearance of the method of Folin and Berghlund<sup>7</sup>), and have found close agreement between the picric acid methods of Benedict and Osterberg, with higher

TABLE I  
COMPARATIVE RESULTS FOR SUGAR IN NORMAL URINE FOUND BY VARIOUS METHODS

CASE	DATE	ACETONE- PICRIC ACID METHOD	MERCURIC NITRATE- PICRIC ACID METHOD	SHAFFER CUPROUS TITRATION METHOD	MYERS PICRIC ACID METHOD
	1920	per cent	per cent	per cent	per cent
S.	11/23-4	0.041			0.082
B.	11/22-3	0.075			0.106
L. A. K.	11/28-9	0.040			0.066
L. A. K.	11/29-30	0.021			0.038
L.	11/28-9	0.022			0.037
P.	11/30-12/1	0.031			0.048
E. L.	11/30-12/1	0.044			0.093
	1921				
C. G.	1/14-15	0.044			0.063
C. G.	1/16-17	0.040			0.052
V. D.	1/18-9	0.114			0.132
V. D.	1/19-20	0.081			0.093
T. A.	5/4-5	0.131	0.123	0.072	
T. A.	5/6-7	0.124	0.120	0.108	
T. A.	5/7-8	0.081	0.089	0.050	
L. N.	5/3-4	0.417	0.485	0.460	
F. H.	5/25-6	0.057	0.048	0.027	
F. H.	5/27	0.017	0.020	0.008	
L. A. K.	5/26-7	0.033	0.029	0.011	
N. B. M.	6/2-3	0.055	0.044	0.019	
C. H.	3/15	0.244	0.290		
C. H.	3/15	0.086	0.088		
J. P.	3/14-5	0.176	0.173		
C. H.	3/20-1	0.037	0.046		
M. K.	3/20-1	0.041	0.042		
M. F. M.	3/20-1	0.097	0.104		

results by Myers' and lower results by Shaffer's method. (Table I.) The last named determination is based on the reduction of copper sulphate by the sugar (after removal of interfering substances with mercuric nitrate) and the iodometric titration of the cuprous oxide. Shaffer and Hartmann have shown that this gives lower results than the mercuric nitrate picric acid method of Benedict and Osterberg. Myers' method depends on the precipitation of creatinine from the urine by saturation with picric acid, and the reduction of an alkaline picrate solution by the sugar in the urine. It is probable that in some cases the removal of creatinine is not so complete as in others, and this would account for the irregularity of the higher figures obtained for sugar by this procedure.

Comparison was made also between the results obtained by the acetone-picric acid method and the Benedict<sup>8</sup> qualitative copper test for sugar. The qualitative copper test is sensitive to slightly less than 0.1 per cent reducing sugar in urine. It is seen in Table II that if the specific gravity of a normal urine is high, i.e., if the urine is concentrated, a higher percentage of sugar is necessary to give even a slight reduction of the copper reagent, than if the urine is more dilute. This is probably due to the interfering action of certain urinary constituents present in larger amount in the concentrated urines.

TABLE II  
COMPARISON OF THE ACETONE-PICRIC ACID METHOD WITH THE BENEDICT QUALITATIVE COPPER TEST

CASE	DATE	URINE				
		VOLUME	SP. GR.	REDUCING SUGAR		
				ACETONE-PICRIC ACID METHOD		BENEDICT QUALITATIVE TEST
	(1920)	c.c.		per cent	grams	
R. B.	12/17-18	1560	1013	0.030	0.47	negative
L. M.	10/27-8	1190	1015	0.048	0.57	negative
C.	11/4-5	2000	1012	0.047	0.97	negative
W.	12/3-4	1260	1019	0.068	0.86	negative
T. B.	11/21-2	720	1025	0.090	0.65	negative
	(1921)					
L. H.	3/30-1	1570	1021	0.099	1.50	very sl. +
L. H.	3/31-4/1	1350	1023	0.098	1.32	very sl. +
L. H.	4/1-2	2180	1017	0.121	2.64	+
A. B.	3/23-4	1000	1020	0.107	1.07	very sl. +
McL.	1/30-1	780	1033	0.108	0.85	very sl. +
McL.	1/28-9	690	1034	0.136	0.94	very sl. +
B. J.	6/18-9	300	1026	0.140	0.42	sl. +
D.	1/25-6	600	1024	0.162	0.97	sl. +
L. E.	2/17-8	730	1028	0.122	0.89	sl. +
M. D.	4/15-6	600	1025	0.117	0.70	sl. +
T. B.	4/6-7	420	1029	0.198	0.83	+
M. C.	6/16-7	690	1016	0.290	2.00	+
M. C.	6/17-8	1110	1020	0.260	2.90	+
M. C.	6/19-20	1420	1013	0.300	4.30	+
C. O.	5/4-5	250	1031	0.265	0.66	+
C. O.	5/5-6	250	1039	0.303	0.76	++
L. N.	5/2-3	1188	1016	0.361	4.16	++
L. N.	5/3-4	1015	1019	0.417	4.20	++
L. N.	5/8-9	960	1019	0.316	3.04	++

That the normal sugar output is remarkably constant on an unvarying diet is shown in Table III, in which our daily findings in a few cases are recorded. It is seen also that a low carbohydrate diet results in a lower daily sugar excretion than a medium or high carbohydrate diet. These facts are in harmony with the findings of Benedict<sup>2</sup> and Folin and Berglund.<sup>9</sup>

The effect of the ingestion of food on the hourly sugar output is shown in one case in Table IV. While the hourly sugar excretion on the morning of December 15, 1920, was 37 gm., after a breakfast containing about 60 grams of carbohydrate, the output rose gradually to 59 mg. per hour; it fell to 34 mg. before lunch and rose after lunch to 76 mg. hourly. On March 2, 1921, during the hour before dinner, 26 mg. of sugar were excreted; after



TABLE III  
THE EFFECT OF DIET ON THE DAILY SUGAR EXCRETION

DATE	CASE	SEX	DIAGNOSIS	URINE			DIET—HIGH, MEDIUM, OR LOW CARBOHYDRATE
				VOLUME c.c.	CREATININE grams	REDUCING SUGAR per cent	
(1920) 11/15-6	T.	♀	Undernutrition, headaches, neurassthenia.	1536	0.933	0.039	Medium
11/16-7	"			1440	0.997	0.042	"
11/17-8	"			1600	0.875	0.034	"
(1921) 3/30-1	M. B.	♀	Dyspitarism, dysthyroidism.	1560	1.310	0.047	Medium
3/31-4/1	"			1580	1.320	0.048	"
3/23-4	M. K.	♀	Psychasthenia, hypotonia.	1710	1.120	0.047	Medium
3/24-5	"			1380	1.140	0.059	"
4/8-9	S. B.	♂	Gastric neurosis.	1320	1.810	0.083	Medium
4/9-10	"			960	1.890	0.125	"
6/17-18	J. S.	♂	Essential hypertension.	1990	1.360	0.062	Medium
6/19-20	"			1780	1.350	0.071	"
2/28-3/1	H. C.	♀	Normal.	1089	1.101	0.059	Sl. low
10/20-1	"			1520	1.095	0.039	Sl. low
10/19-20	"			1330	1.095	0.065	Medium
(1922) 4/4-5	"			1925	1.115	0.040	Medium
4/5-6	"			1880	1.250	0.041	Medium
(1920) 12/14-15	"			1747	....	0.065	High
(1922) 3/26-27	H. N. L.	♂	Chronic myocarditis.	550	0.599	0.012	Very low
4/13-14	"			1020	0.630	0.060	Medium
5/12-13	K. L.	♂	Chronic interstitial nephritis.	1820	0.650	0.100	High
6/8-9	"			1520	0.610	0.060	Low

dinner, and later after additional food was eaten, the hourly output rose to 84 mg.

Our plan was to determine the amount of reducing substance (sugar) in twenty-four hour urine specimens of normal individuals and hospital patients, using the technic of the new acetone-picric acid method of Benedict and Osterberg. In all cases the sugar was determined in several twenty-four hour specimens, and the accuracy of the urine collections was checked by determinations of the daily creatinine output. Note was made of the diets of the individuals and these were classified according to carbohydrate content. The daily output of sugar was comparatively constant when the diet contained about the same amount of carbohydrate each day; accordingly, in order to save space, the daily urine volumes and amounts of sugar excreted by each individual were averaged for Table V; the average figures there shown are much the same as the figures obtained for each of the individual days.

It was found that in twelve normal adults, from 0.62 to 1.23 grams of reducing sugar on the average were excreted in twenty-four hours. The average figure for the twelve cases was 0.96 gram. Neuwirth,<sup>10</sup> using the same method<sup>4</sup> of sugar determination in studying twenty-six normal individuals, has demonstrated a daily excretion of 0.941 gram reducing sugar on the average, with a range of 0.614 to 1.383 grams. Folin and Berglund,<sup>8</sup> using their new colorimetric method<sup>7</sup> for sugar in normal urine, determined the twenty-four hour amounts of sugar in the urine of a normal man. When he was on a diet of meat, eggs and 50 or 200 grams of pure glucose daily for eight days, the daily amount of reducing sugar was between 0.543 and 0.980 gram; when he was on a mixed diet, including starches, for six days, the twenty-four hour excretion of reducing sugar was from 0.885 to 1.650 grams. The average daily figure for the six days on a mixed diet was 1.203 grams reducing sugar. Folin and Berglund showed also that there is an increase in the reducing sugar in normal urine after hydrolysis, confirming the observation previously made by Benedict.<sup>11</sup>

We found an average daily excretion of 0.42 to 1.07 grams of reducing sugar in twelve patients diagnosed as neurasthenics. In twenty-four patients suffering from conditions associated with constipation, the average range was from 0.41 to 1.80 grams reducing sugar daily, with the exception of one man (M. H-e. Table V) who excreted 3.85 grams reducing sugar in one day. No reason was found for this relatively high sugar excretion, either in the diet or from the history. In six cases of hyperthyroidism an average of 0.46 to 1.61 grams reducing sugar were excreted daily. Four patients with dyspituitarism excreted daily from 0.69 to 1.18 grams reducing sugar on the average, while a patient suffering from hypothyroidism excreted 0.40 gram sugar, after a low carbohydrate diet. A woman having myxedema excreted on the average 0.62 gram reducing sugar daily. It has been shown by various investigators<sup>12</sup> that a high fasting blood sugar and low carbohydrate "tolerance," or high and delayed blood sugar curve after glucose ingestion, may exist in hyperthyroidism, and a low fasting blood sugar and high "tol-

TABLE IV

THE EFFECT OF FOOD INGESTION ON THE HOURLY EXCRETION OF SUGAR  
CASE C. (NORMAL)

HOUR	FOOD	URINE				SUGAR
		VOLUME	SP. GR.	REDUCING SUGAR		CALCULATED HOURLY
(12/14/20)	8 A. M.	C.C.		PER CENT	MGS.	MGS.
A. M.	Breakfast					
9-11	(45 g. CHO)	100	1022	0.058	58	29
11-12		50	1021	0.055	28	
P. M.	1 P. M.					
12-1	Lunch	34	1024	0.064	22	
1-2	(50 g. CHO)	34	1024	0.073	25	
2-3		33	1027	0.099	33	
3-4		39	1028	0.091	35	
4-5		42	1029	0.083	35	
5-6	6 P. M.	24	1032	0.094	23	
	Dinner					
6-7	(120 g. CHO)	40	1030	0.101	41	
7-8		47	1031	0.103	49	
8-9.30	9 P. M.	106	1030	0.086	91	61
	Candy					
9.30-10.30	(60 g. CHO)	69	1028	0.105	72	
10.30-8 A. M.	8 A. M.	615	1015	0.057	352	37
	Breakfast					
(12/15/20)	(60 g. CHO)					
8-9		50	1018	0.083	42	
9-10		101	1017	0.058	59	
10-11		128	1016	0.043	56	
11-12	12.15 P. M.	104	1015	0.033	34	
P. M.	(50 g. CHO)					
12-1	1 P. M.	72	1019	0.058	42	
	Lunch					
1-4	(40 g. CHO)	326	1015	0.070	229	76
4-5		65	1018	0.086	56	
Total	About					
32	425 g. CHO	2079		0.065	1382	
2/28/21						
A. M.	Breakfast					
	(40 g. CHO)					
9-1	1 P. M.	335	1021	0.043	139	35
P. M.	Lunch					
1-2	(65 g. CHO)	70	1018	0.038	27	
2-4		83	1021	0.061	51	26
4-5		26	1029	0.110	29	
5-6		33	1022	0.083	27	
	6 P. M.					
6-7	400 c.c. H <sub>2</sub> O	60	1023	0.082	49	
7-8		49	1022	0.077	38	
8-9		77	1018	0.054	42	
9-10	9.30 P. M.	57	1017	0.045	26	
	Lunch					
10-10.30	(20 g. CHO)	14	q. n. s.	0.069	9.6	19
10.30-8		270	1017	0.073	196	21
	8 A. M.					
3/1/21	Breakfast					
A. M.	(40 g. CHO)					
8-9		15	q. n. s.	0.103	16	
9-10		15	q. n. s.	0.122	18	
10-11		50	1021	0.082	41	
11-12		37	1022	0.078	29	
Total	About					
27	165 g. CHO	1191		0.062	737.6	

TABLE IV—CONT'D

THE EFFECT OF FOOD INGESTION ON THE HOURLY EXCRETION OF SUGAR  
CASE C. (NORMAL)

HOUR	FOOD	URINE				SUGAR CALCULATED HOURLY
		VOLUME	SP. GR.	REDUCING SUGAR		
3/2/21	8 A. M.	C.C.		PER CENT	MGS.	
A. M.	Breakfast					
9-11	(55 g. CHO)	108	1024	0.082	88	44
11-12	12 M.	53	1023	0.065	34	
	Milk					
12-1	(10 g. CHO)	31	1027	0.072	22	
1-2	1 P. M.	45	1027	0.077	35	
2-3	Lunch	53	1027	0.078	41	
	(65 g. CHO)					
3-4		54	1025	0.064	35	
4-5	4 P. M.	185	1015	0.028	52	
5-6	400 c.c. H <sub>2</sub> O	105	1015	0.025	26	
	6 P. M.					
6-7	Dinner	72	1016	0.036	26	
	(55 g. CHO)					
7-8	7 P. M.	56	1016	0.051	29	
8-9	Fruit	63	1015	0.085	54	
	(25 g. CHO)					
9-10	8 P. M.	175	1010	0.048	84	
	Fruit					
	(15 g. CHO)					
Total	About					
13	225 g. CHO	1000		0.053	526	

erance'' are often seen in hypothyroidism and dyspituitarism. It is interesting to note that no great departure from normal daily sugar excretion was seen in our cases of these diseases.

In our series of thirteen patients with vascular hypertension, there was a daily average sugar excretion of 0.44 to 1.12 grams. Five patients with cardiac disorders excreted from 0.51 to 0.71 gm. sugar daily. In ten cases of nephritis we found an average range from 0.61 to 1.89 grams. Although in cases of severe nephritis a mild hyperglycemia is generally present, no essential change from normal sugar excretion was found in these cases. It appears that there is no lowering in the excretion of sugar in nephritis.

Carbohydrate metabolism may be disturbed in some way in arthritis, according to Pemberton.<sup>13</sup> We studied sugar excretion in seven cases of arthritis and found that the range of daily sugar excretion was between 0.44 and 1.39 grams. In three cases of rheumatism, from 0.80 to 1.23 grams of sugar were excreted daily.

It has been shown by various investigators that there is a hyperglycemia in carcinoma. We found no variation from the normal sugar excretion in six cases of abdominal carcinoma, with an average range of 0.62 to 0.81 gm. sugar daily.

In a series of hospital cases representing a variety of pathologic conditions, in which were included disorders of the stomach, uterus and gall bladder, and such diseases as eczema and encephalitis lethargica, no departure from normal sugar excretion was noted. The case of A. N-1. (Table V), a



TABLE V  
AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE YEARS	WEIGHT KILOGS	DIET,* LOW, MEDIUM OR HIGH CHO	NUMBER OF DAILY DETNS AVERAGED	URINE			DIAGNOSIS
							VOLUME	REDUCING SUGAR	GRAMS	
							C.C.	PER CENT		
2-21	H. C.I.	♀	25	52	Sl. low	2	1305	0.048	0.62	Normal
	H. C.I.				Medium	3	1711	0.047	0.80	
	H. C.I.				High	1	1747	0.065	1.14	
6-22	H. De K.-f.	♀	24	53	Low	1	465	0.140	0.65	Normal (Father had diabetes)
5-22	G. P.-n.	♂	24	55	Medium	3	1510	0.055	0.83	Normal
4-22	L. M.-r.	♀	45	61	Medium	4	1169	0.074	0.87	Normal
6-22	M. W.-b.	♀	24	70	Medium	1	785	0.113	0.89	Normal
12-21	V. M.-s.	♂	38	77	High	3	838	0.115	0.99	Normal
	C. R.-h.	♀	17	48	Medium	2	915	0.113	1.03	Normal
4-22	F. T.-n.	♀	35		Medium	1	1910	0.054	1.03	Normal
12-21	H. B.-e	♀	25	52	Medium	1	1560	0.068	1.06	Normal
3-21	A. S.-s.	♀	35	70	High	2	2600	0.044	1.14	Normal
12-21	D. K.-l.	♂	40	70	Medium	1	1010	0.118	1.19	Normal
	M. C.-h.	♀	27	64	High	3	725	0.170	1.23	Normal
10-20	J. L. F.-y.	♀	35		Medium	1	890	0.046	0.41	Anemia
1-21	Me L.-n.	♀	17	55	Medium	6	840	0.090	0.76	Undernutrition
2-21	W.-y.	♀	25	63	Medium	1	1440	0.063	0.90	Postoperative convalescence
2-21	M.-e.	♀	41	61	Medium	1	1020	0.041	0.42	Psychasthenia
3-21	B. A. S.-s.	♀	60	39	Medium	3	710	0.062	0.44	Psychosis
3-21	C. H.-y.	♀	57	50	Medium	3	1120	0.052	0.58	Psychasthenia, hypotonia
11-20	T.-x.	♀	44	56	Medium	3	1525	0.039	0.60	Neurasthenia, undernutrition
12-20	A.-h.	♀	38	61	Medium	4	1031	0.060	0.62	Neurasthenia, optic neuritis
3-21	M. K.-p.	♀	57	73	Medium	3	1670	0.047	0.80	Psychasthenia, hypotonia
12-20	C.-e.	♀	57	70	Medium	2	1230	0.066	0.81	Neurasthenia
	B.-d.	♀	61	61	Medium	2	1560	0.052	0.82	Neurasthenia
12-20	W.-r.	♀	62	68	Medium	3	1250	0.066	0.83	Neurasthenia, undernutrition
2-21	E.-n.	♂	53	48	Medium	3	1190	0.072	0.86	Psychosis, anemia
5-21	P. C. B.-e.	♂	31	68	Medium	3	1480	0.063	0.94	Hypotonia, psychasthenia
3-21	A. J. B.-n.	♀	23	68	Medium	1	1000	0.107	1.07	Neurasthenia
11-20	L.-y.	♀	61	68	Medium	1	1810	0.022	0.41	Constipation, psychoneurosis
12-20	G.-n.	♂	64	64	Medium	1	1620	0.028	0.46	Constipation, diverticuli coli

\*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

TABLE V—Continued  
AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE  YEARS	WEIGHT  KILOGS	DIET,* LOW, MEDIUM OR HIGH CHLO	URINE			DIAGNOSIS
						NUMBER OF DAILY DETNS AVERAGED	VOLUME	REDUCING SUGAR	
							C.C.	PER CENT	GRAMS
5-21	E. R-n.	♀	48	54	Medium	3	760	0.066	0.50
3-21	R. B-c.	♀	26	50	Medium	3	1360	0.037	0.50
1-21	N-t.	♀	37	45	Medium	3	1120	0.055	0.62
11-20	B-r.	♂	44	63	Medium	1	900	0.075	0.67
3-21	M. F. M-c.	♀	14	50	Medium	3	942	0.071	0.67
4-21	M. F-d.	♀	34	64	Medium	2	1740	0.041	0.71
1-21	E. L-c.	♂	38	66	Medium	7	1724	0.041	0.71
3-21	F. S-l.	♀	60	60	Medium	1	1650	0.047	0.77
12-20	R. G-k.	♀	40	64	Medium	4	1764	0.045	0.80
3-21	W. G. L-d.	♀	60	64	Medium	1	945	0.087	0.83
4-21	H. E. V-n.	♂	60	77	Medium	6	2070	0.041	0.85
4-21	J. A-r.	♀	59	63	Medium	2	1140	0.077	0.88
1-21	G-u.	♀	38	55	Medium	4	2260	0.040	0.91
2-21	P. H. S-h.	♀	46	64	Medium	1	1600	0.096	0.96
11-20	C-t.	♀	42	60	Medium	1	2000	0.048	0.97
1-21	D-l.	♀	35	53	Medium	5	1620	0.060	0.98
11-20	W-g.	♀	43	57	Medium	2	1455	0.069	1.01
4-21	H. S. McK-c.	♂	30	64	Medium	3	860	0.118	1.01
4-21	V. E. M-y.	♂	50	66	Medium	3	1336	0.093	1.24
1-22	J. G-s.	♂	45	73	High	5	1418	0.119	1.69
3-21	L. F. H-z.	♂	33	63	High	3	1700	0.106	1.80
12-20	M. H-c.	♂	46	66	Medium	1	2790	0.138	3.85
3-21	W. H-h.	♀	24	50	Medium	1	550	0.083	0.46
2-21	H. R-n.	♂	18	54	Medium	6	964	0.064	0.62
6-21	R. M-s.	♀	22	49	Medium	2	660	0.130	0.86
2-21	G.	♀	40	60	Medium	7	1377	0.064	0.88
5-21	T. A-n.	♀	27	47	Medium	9	842	0.116	0.98
1-22	L. G-g.	♂	36	50	High	7	1683	0.096	1.61

\*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHLO diet about 250 to 300 gm., and high CHLO diet over 300 gm. For children the amounts are smaller.

TABLE V—Cont'd  
AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE YEARS	WEIGHT KILOS	DIET,* LOW, MEDIUM OR HIGH CHO	NUMBER OF DAILY DETENS AVERAGED	URINE			DIAGNOSIS
							VOLUME C.C.	REDUCING SUGAR PER CENT	GRAMS	
4-21	R. C-c.	♀	31	53	Medium	2	1080	0.077	0.83	Dysthyroidism, neurasthenia
3-21	M. B-s.	♀	19	78	Medium	2	1570	0.047	0.74	Dysthyroidism, dyspituitarism
11-20	B-t.	♀	63	70	Medium	1	1620	0.043	0.69	Dyspituitarism, undernutrition, migraine
11-20	Mc. R-s.	♀	46	91	Medium	2	1845	0.057	1.05	Dyspituitarism, hypotonia, colitis
4-21	W. E-n.	♀	43	89	Medium	1	1620	0.073	1.18	Dyspituitarism, obesity
6-21	B. J-n.	♀	58	86	Low	3	460	0.087	0.40	Hypothyroidism, obesity, hypertension
1-22	L. B-k.	♀	52	86	Medium	5	1060	0.058	0.62	Myxedema
11-20	P-y.	♀	54	71	Medium	1	1440	0.031	0.44	Vascular hypertension, neurasthenia
8-21	G. S-l.	♀	52	86	Low	2	345	0.133	0.46	Essential hypertension
2-21	E. H-r.	♀	73	57	Medium	7	1046	0.049	0.51	Essential hypertension
1-21	F-n.	♀	50	50	Medium	2	1770	0.033	0.58	Vascular hypertension, neurasthenia, headaches
4-21	M. D-g.	♀	56		Medium	16	815	0.079	0.59	Cardiac hypertrophy, hypertension
12-20	S-t.	♀	60	64	Medium	4	990	0.060	0.59	Vascular hypertension, colitis, neurasthenia
4-21	M. D-s.	♀	23	53	Medium	13	650	0.091	0.59	Hypertension, hysteria
2-21	E. F-n.	♀	48	84	Medium	6	1870	0.038	0.71	Essential hypertension
11-20	B-r.	♀	40	63	Medium	1	1680	0.054	0.91	Vascular hypertension, colitis
4-21	B. Y-r.	♂	53	69	Medium	7	1494	0.062	0.93	Essential hypertension
2-21	L. F-r.	♂	36	68	Medium	8	765	0.128	0.98	Essential hypertension
4-21	B. de C-r.	♂	40	60	Medium	2	2100	0.045	0.99	Vascular hypertension, neurasthenia, constipation, migraine
6-21	J. S-r.	♂	41	70	Medium	3	1790	0.062	1.12	Essential hypertension
5-21	F. H-l.	♀	30	54	Medium	4	1037	0.080	0.83	Migraine, hypotonia
2-21	B. G-d.	♂	27	58	High	9	988	0.062	0.61	Chronic diffuse nephritis
4-21	E. G-e.	♂	23	66	Medium	1	1260	0.052	0.66	Nephritis, secondary contracted kidney
3-21	P. R-o.	♂	41	64	Medium	6	1630	0.042	0.69	Chronic interstitial nephritis
6-21	J. S-r.	♂	17	44	Medium	1	1535	0.046	0.71	Chronic diffuse nephritis, complications
12-20	M-y.	♀	51	59	Medium	5	1626	0.048	0.78	Chronic nephritis, hypertension, cystitis
1-22	J. B-e.	♂	50	52	Medium	9	1329	0.062	0.82	Arteriosclerotic kidneys, cardiac hypertrophy and dilatation

\*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

TABLE V—Cont'd  
AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE YEARS	WEIGHT KILOGS	DIET,* LOW, MEDIUM OR HIGH CHO	NUMBER OF DAILY DETNS AVERAGED	URINE			DIAGNOSIS
							VOLUME	REDUCING SUGAR	PER CENT	
							C.C.	GRAMS		
6-21	J. S-k.	♀	38	63	Medium	4	1119	0.89	0.080	Chronic pyelonephritis
1-22	L. L-o.	♀	55	59	High	4	1325	1.25	0.094	Arteriosclerotic kidneys, hypertension
2-22	J. M-i.	♂	55	68	High	14	2045	1.72	0.084	Arteriosclerotic kidneys, cardiac hypertrophy and dilatation
5-22	S. K-e.	♂	26	60	High	10	1499	1.89	0.126	Chronic interstitial nephritis
6-21	S. B-y.	♀	45	52	Medium	4	537	0.51	0.095	Chronic cardiovascular disease
4-21	M. M-y.	♀	50	51	Medium	9	432	0.55	0.127	Arteriosclerosis, gastritis
11-21	H. N-l.	♂	70	64	Medium	7	925	0.58	0.063	Chronic myocarditis
5-21	C. O-N-l.	♀	52	83	Medium	7	516	0.65	0.126	Auricular fibrillation, edema
3-21	J. F-r.	♂	48	73	Medium	3	1190	0.71	0.060	Auricular fibrillation
6-21	M. H-r.	♂	39	80	Very low	1	455	0.14	0.097	Osteoarthritis
1-21	B-n.	♀	53	66	Medium	2	2250	0.51	0.023	Chronic arthritis, eczema, neurasthenia, constipation
6-21	N. B. M-y.	♀	51	73	Medium	3	960	0.60	0.062	Arthritis
3-21	E. R-	♀	38	—	Low	1	1086	0.67	0.062	Arthritis deformans
7-21	R. C-s.	♂	24	65	Medium	6	757	0.89	0.117	Osteoarthritis
5-21	C. T-r.	♂	49	103	Very low	2	2641	1.07	0.040	Acute arthritis, chronic myocarditis
4-21	H. K-n.	♂	30	62	Medium	12	1013	1.39	0.123	Arthritis deformans, mitral stenosis and insufficiency
3-21	C-l.	♀	39	—	Medium	3	1450	0.80	0.055	Rheumatism
11-20	B-s.	♀	69	—	Medium	1	1560	1.09	0.071	Rheumatism, constipation, vascular hypertension
7-21	H. S-u.	♂	14	37	High	8	1177	1.23	0.105	Rheumatic fever, tonsillitis
1-21	Y-e.	♂	60	45	Medium	3	1980	0.62	0.031	Abdominal carcinoma, anemia
11-20	U. S-d.	♂	77	62	Medium	1	1620	0.67	0.041	Carcinoma of stomach, abdominal metastases
2-21	W. S. M-r.	♀	54	50	Medium	1	470	0.71	0.150	Carcinoma of sigmoid
2-21	F. S-r.	♀	52	76	Medium	1	1430	0.71	0.049	Intrapertoneal carcinoma
12-20	E. W-e.	♀	50	54	Medium	1	1820	0.73	0.040	Carcinoma recti
7-21	A. R-e.	♂	51	57	Medium	3	583	0.81	0.139	Carcinoma of liver and abdomen
3-21	L. A. K-n.	♀	31	61	Medium	10	1153	0.46	0.040	Achylia gastrica, neurasthenia

\*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.



TABLE V—CONT'D  
AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE YEARS	WEIGHT KILOGS	DIET,* LOW, MEDIUM OR HIGH CHO	NUMBER OF DAILY DETNS AVERAGED	URINE			DIAGNOSIS
							VOLUME C.C.	PER CENT	REDUCING SUGAR GRAMS	
12-20	E. Y.	♀	39	63	Medium	2	1110	0.079	0.83	Gastric subacidity, cystitis, endometritis
12-20	R. L.	♂	28	64	Medium	1	1000	0.113	1.13	Gastric hyperacidity, neurasthenia
4-21	S. B. n.	♂	36	73	Medium	2	1140	0.101	1.15	Gastric neurosis
1-21	E. C.	♀	19	59	Medium	2	705	0.086	0.61	Perimetritis, editis, tonsillitis
5-21	J. B. T. d.	♀	44	47	Medium	1	1184	0.058	0.69	Menopause, anemia, menorrhagia
2-21	T. n.	♀	44	51	Medium	19	1835	0.177	0.70	Fibromyoma-uterus, anemia, neurasthenia
1-21	H. r.	♀	33	49	Medium	2	945	0.053	0.50	Cholecystitis, constipation, neurasthenia
12-21	J. B. s.	♂	41	—	Medium	5	2061	0.063	1.30	Hemolytic jaundice
12-20	C. S. d.	♂	54	63	Medium	2	1680	0.053	0.89	Chronic appendicitis, peritoneal adhesions
12-21	H. F. t.	♂	35	—	Medium	5	1137	0.082	1.18	Eczema
1-21	T. Van D. n.	♂	60	63	Medium	3	1560	0.124	1.93	Liver cirrhosis, intestinal stasis
10-20	L. H. M. r.	♀	41	66	Medium	1	1190	0.048	0.57	Middle ear vertigo
1-22	A. N. l.	♂	50	72	Medium	1	8800	0.0102	0.90	Optic atrophy,—question of brain tumor
4-21	T. B. e.	♂	28	54	Medium	2	570	0.130	0.74	Encephalitis lethargica
1-22	L. C. y.	♀	31	59	Low	5	914	0.158	1.44	Lactosuria, secondary anemia
1-22	B. T. y.	♀	11	39.5	Very low	4	391	0.089	0.35	Mild diabetes, (urine "sugar-free")
3-22	A. B. n.	♂	6	19.5	Very low	9	1315	0.039	0.51	Diabetes mellitus, (urine "sugar-free")
12-21	M. S. d.	♀	20	61	Very low	7	1150	0.049	0.57	Diabetes mellitus, (urine "sugar-free")
1-22	S. F. n.	♀	53	—	Very low	4	1185	0.053	0.63	Diabetes mellitus, (urine "sugar-free")
12-21	S. S. z.	♀	54	—	Very low	2	750	0.100	0.75	Diabetes mellitus, (urine "sugar-free")
12-21	V. L. n.	♀	59	77	Very low	2	1175	0.071	0.84	Diabetes mellitus, (urine "sugar-free")
11-21	S. S. l.	♂	61	53	Very low	10	1135	0.075	0.85	Arteriosclerosis, diabetes mellitus, (urine "sugar-free")
9-21	T. M. s.	♀	16	45	Medium	2	1530	0.061	0.93	Blood sugar of 0.157 per cent

\*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

TABLE V—CONT'D  
AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE YEARS	WEIGHT KILOGS	DIET, * LOW, MEDIUM OR HIGH CHO	NUMBER OF DAILY DETNS AVERAGED	URINE			DIAGNOSIS
							VOLUME C.C.	PER CENT	REDUCING SUGAR GRAMS	
3-21	P. C-r.	♂	17	68	Medium	3	1370	0.133	1.83	Diabetes, vascular hypertension Cardiospasm, (one sister has diabetes)
6-21	M. C-t.	♂	47	54 Lost 27 kilos in last year	Medium	3	1073	0.289	3.10	
5-21	L. N-l.	♀	45	68	Medium	5	1221	0.281	3.43	Chronic interstitial nephritis, history of diabetes
11-21	E. Mc C-y.	♀	25	48	Medium	3	910	0.585	5.32	
1-22	J. C-k.	♀	58	—	Very low	1	575	3.100	17.85	Diabetes mellitus, (urine contains sugar)
					Very low	1	1200	0.101	0.87	
1-22	M. A s.	♂	45	68	Very low	1	1080	3.400	36.80	Diabetes mellitus, (urine contains sugar)
					Very low	1	782	0.100	0.78	
11-21	S. N-r.	♂	43	61	Very low	1	1710	1.520	26.00	Diabetes mellitus
12-21	R. C-y.	♂	42	50	Very low	1	2160	1.500	32.4	
3-22	S. L-y.	♂	9	23	Very low	1	2220	1.800	39.9	Diabetes mellitus
7-21	H. M-l.	♀	5.5	16	Low	1	235	0.049	0.12	
7-21	J. D-a.	♀	3.8	12.5	Medium	4	275	0.047	0.13	Cardiac insufficiency, edema Empyema—old
6-21	M. Mc G-h.	♀	2.5	13.5	Medium	1	105	0.182	0.19	
7-21	J. C-y.	♂	6	20	Medium	1	630	0.032	0.20	Chronic arthritis Foreign body in right bronchus
6-21	J. M-o.	♂	4.8	12	Medium	7	219	0.110	0.24	
7-21	E. K-a.	♀	3	12	Medium	1	290	0.114	0.33	Erythema multiformis Chronic interstitial nephritis
6-21	S. B-d.	♂	5.7	18.8	Medium	8	390	0.090	0.35	
7-21	C. M-o.	♀	6	20	Medium	9	508	0.075	0.38	Chorea, endocarditis Asthma, constipation
4-21	O. H. S-s.	♂	7	—	Medium	5	1064	0.037	0.39	
3-21	J. P-s.	♀	9	23	Medium	6	373	0.115	0.43	Chronic parenchymatous nephritis

\*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

fifty year old man suffering from optic atrophy, is interesting since it shows that even with the enormous excretion of urine in twenty-four hours,—8800 c.c.,—the amount of sugar excreted was normal, 0.90 gram, in a concentration of 0.0102 per cent.

The daily sugar excretion was studied in ten children suffering from various disorders, and an average range from 0.12 to 0.43 gm. was found. Smaller body weight and lower food intake may be the factors responsible for the lower excretion of sugar by children than by adults.

Increased sugar excretion in diabetes mellitus is a well-known phenom-

TABLE VI

THE EXCRETION OF FERMENTABLE AND NONFERMENTABLE SUGAR IN DIABETES

CASE	DATE	VOLUME	URINE						DIAGNOSIS
			QUALITA- TIVE BENEDICT TEST	REDUCING SUGAR				NONFER- MENT- ABLE SUGAR	
				BEFORE FERMENTATION		AFTER FERMENTATION			
	1922	C.C.		PER CENT	GRAMS	PER CENT	GRAMS	PER CENT OF TOTAL	
B. T-y.	1/ 8-9	460	Sl. +	0.147	0.68	0.089	0.41	60.3	Mild diabetes
	1/ 9-10	460	Neg.	0.077	0.35	0.054	0.25	70.6	
S. F-n.	1/ 8-9	1400	Neg.	0.065	0.91	0.036	0.51	56.0	Diabetes mellitus
J. C-k.	1/ 6-7	900	+	0.260	2.34	0.087	0.78	33.3	Diabetes mellitus
	1/ 7-8	575	Strongly +	3.100	17.85	0.107	0.61	3.4	
	1/ 8-9	800	Sl. +	0.220	1.76	0.067	0.54	30.7	
	1/ 9-10	600	Sl. +	0.167	1.00	0.076	0.46	46.0	
	1/10-11	1200	Neg.	0.101	0.87	0.058	0.69	79.3	
M. A-s.	1/ 8-9	1080	Strongly +	3.400	36.80	0.098	1.07	2.9	Diabetes mellitus
R. C-y.	1/ 5-6	2400	Strongly +	1.500	36.00	0.034	0.82	2.3	Diabetes mellitus
	1/ 6-7	2480	Strongly +	2.000	49.60	0.038	0.95	1.9	
	1/ 7-8	2300	Strongly +	2.500	57.50	0.036	0.83	1.4	
	1/ 8-9	2550	Strongly +	3.000	76.50	0.037	0.95	1.2	
I. C-y.	1/ 5-6	800	+	0.196	1.57	0.196	1.57	100.0	Lactosuria, 12 weeks after parturition
	1/ 6-7	1300	+	0.156	2.03	0.105	1.36	67.0	
	1/ 7-8	850	+	0.230	1.95	0.104	0.88	45.0	
	1/ 9-10	620	Sl. +	0.133	0.83	0.110	0.68	82.0	
	1/10-11	1000	Neg.	0.081	0.81	0.070	0.70	86.4	

enon. In our series in Table V we show the sugar excretion in a few diabetic patients. The range shown there is from 1.83 to 39.90 gm. reducing sugar daily, when the urine gives a positive reaction with Benedict's qualitative copper reagent. Of the diseases we have studied, diabetes mellitus is the only one in which we have noted sugar excretions markedly above the normal. The case of M. C-t. (Table V), a forty-seven year old man suffering from eardiospasm, is of interest. In the last year this patient lost 27 kilos in body weight. He has one sister who has diabetes. On June 9, 1921, he showed a fasting blood sugar of 0.145 per cent, and on June 17 a high normal blood sugar of 0.120 per cent, while for three days after June 17 he showed

an average daily sugar excretion of 3.10 gm. on a medium carbohydrate diet. The average sugar concentration in the urine was 0.289 per cent; these urine specimens gave positive Benedict qualitative tests.

Determinations of the sugar in the twenty-four hour urines of ten diabetic patients were made when the urines were "sugar-free," i.e., gave a negative result with Benedict's qualitative copper reagent. It is seen (Table V) that under these conditions the sugar output is practically normal, showing an average range from 0.35 to 0.93 gm. daily. This observation is in harmony with the findings of Felsher,<sup>11</sup> who showed that as long as the diet of diabetic patients contains a total glucose equivalent below a certain limit (which varies with the individual), the quantities of sugar excreted are not greater than those excreted by normal individuals. She showed also that after this limit is passed, further addition to the diet causes rapid and sudden increases in the sugar excretion.

In Table VI are shown the amounts of reducing sugar excreted by several diabetic patients, as determined before and after fermentation with yeast. It is seen that when the urine gave a negative reaction with Benedict's qualitative reagent, the percentage of nonfermentable reducing sugar was high, 56 to 79.3 per cent of the total reducing sugar. When there was only a slightly positive reaction to Benedict's reagent, the nonfermentable sugar was 30.7 to 60.3 per cent of the total sugar. On the other hand, when there was a strongly positive reaction to Benedict's reagent, the nonfermentable sugar represented only 1.2 to 3.4 per cent of the total, since by far the largest part of the reducing sugar excreted was glucose. The last case in Table VI was that of a woman, I. C-y., who had lactosuria. The excretion of lactose decreased until finally the urine was negative with Benedict's qualitative test. The percentages of nonfermentable sugar in this case are not unlike those observed in two normal men by Benedict, Osterberg and Neuwirth.<sup>2</sup>

#### SUMMARY

A comparative study of different methods for the determination of sugar in normal urine has been made. By the use of one of these methods, that of Benedict and Osterberg,<sup>4</sup> it was shown that there is an increase in the amount of reducing sugar excreted hourly after meals. It was found that a diet rich in carbohydrate increases the amount of sugar excreted over that on a low carbohydrate diet.

The daily excretion of reducing sugar in the urine was determined in 12 normal individuals and 140 hospital cases. In none of the diseases studied, except diabetes mellitus, is there any marked variation from the normal sugar excretion on ordinary diets. Moreover, in diabetes when by dietary regulation the patient is rendered "sugar-free," the daily amount of sugar excreted is practically normal.

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## THE ETIOLOGY OF GALLSTONES\*

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IN DISCUSSING a problem as old as the subject of gallstones, it is quite essential that one do so with caution and due modesty. It is a problem that has occupied the minds of scientific men for centuries because very early in the practice of medicine, physicians were called upon to treat this baffling ailment, and render succor to those so afflicted. The following communication is offered with the sincere hope that it may be of aid in reaching a more definite conclusion as to the etiology and alleviation of this very common disease.

Many theories and predisposing causes have been suggested to explain the formation of gallstones. A few of the important factors already suggested will be briefly reviewed before entering into the subject-matter of this paper.

It has been noted that gallstones are more common in women than in men, and that it is a disease confined largely to middle or advanced life. Likewise we know that it occurs more frequently in individuals of sedentary habits than in active persons engaged in hard physical labor.

Frerichs<sup>1</sup> suggested quite early that the essential factor for the formation of stones was a stasis or stagnation of the bile. Somewhat later the popular theory of the infectious origin of gallstones was advanced by Naunyn.<sup>2</sup> This theory was championed by such men as Gilbert, Girode, Dufort and Chiari.

Following the contribution of Naunyn, Cushing<sup>3</sup> advanced the theory that the essential conditions necessary for stone formation were stagnation of the bile or obstruction with the presence of infection. This theory is really a combination of the ideas of Frerich and Naunyn. An interesting and important contribution to the study of gallstones was later made by Kramer<sup>4</sup> who showed that filtered normal bile to which peptone broth was added and infection then introduced, underwent changes that were strikingly like those seen in pathologic gall bladders. He noted that under these conditions, cholesterol pigment and calcium salts settled out in the course of a few months and con-

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cretions of the same composition as gallstones formed. This work was subsequently confirmed by Baemeister.<sup>5</sup> Brockbank<sup>6</sup> then showed that bile obtained postmortem if admixed with desquamated bladder cell, on standing, showed a deposit of cholesterin. Sterile normal bile shows the same phenomenon after standing for prolonged periods of time. Aschoff<sup>7</sup> states that pure cholesterin stones are always obtained from bladders showing distention but never occur in chronically inflamed organs. He noted that the bile in these cases was frequently sterile. Pure cholesterin stones are very rare, as in the vast majority of cases, slight amounts of mucoid matter, pigment and salts, are present.

Adami<sup>8</sup> suggests that the probable steps in gallstone formation are:

1. Infection (*B. coli*, pyogenic cocci, *B. typhosus*).
2. Stagnation of bile.

As a result of this stagnation, an absorption of alkaline substances takes place with the production of an acid bile. This leads to a catarrh with an outpouring of mucus in which the pigments and salts are precipitated. He states that stones may be found anywhere in the biliary passages. In my studies up to the present time I have never encountered an acid bile and this explanation does not seem logical since we would expect in such cases to find gallstones composed largely of bile salts, since they are precipitated in an acid medium. Bile after prolonged stagnation and putrefaction undergoes an ammoniacal change and often becomes intensely alkaline in reaction.

The chief forms of stones encountered in clinical practice are those composed of cholesterin alone, or cholesterin and pigment and  $\text{CaCO}_3$  (laminated gallstone) and the common gallstone which is made up largely of cholesterin and bilirubin calcium with small amounts of  $\text{CaCO}_3$ . The cholesterin gravel which consists of fine deposits of amorphous cholesterin is probably an early stage of stone formation.

Another form is described as the pure bilirubin calcium calculi or black bile gravel lying in a mucoid bile. These are soft stones embedded in mucus, containing calcium salts, bilirubin and a trace of cholesterin. This latter type, unfortunately, I have not encountered.

An extremely rare type is also described as the calcium carbonate stones. These are found chiefly in the herbivora but rarely ever in man.

To the above etiologic factors may be added the reduction or absence of bile salts in the bile. This may be brought about by infection, or hepatic insufficiency. Mayo-Robson<sup>9</sup> suggests this factor as an etiologic possibility. The following work deals largely with this latter change and suggests the various findings which bring about this disturbed bile salt secretion. The importance of the bile salts in the production of gallstones will be elaborated and an attempt made to correlate these findings with what takes place in the body in cholelithiasis.

While performing certain routine tests on bile obtained at operation, after cholecystostomy and at postmortems, I was struck with the striking dissimilarities in various specimens which to outward appearances were alike. It occurred to me that it would be of interest in connection with urinary

determinations on cases of cholelithiasis, to likewise at the same time determine the nature of the bile secreted by the liver. The cases here presented are all cases of gallstones. Cystic duct obstruction was usually present, while in a few cases there occurred also common duct obstruction with jaundice.

In all cases the urine was examined prior to examination, and the urine and bile daily examined for fourteen to sixteen days after operation. As previously reported,<sup>10</sup> all such cases show an increase in bile salts and pigment in the urine before operation. As a result of the obstruction to the

#### NONCALCULOUS CHOLECYSTITIS

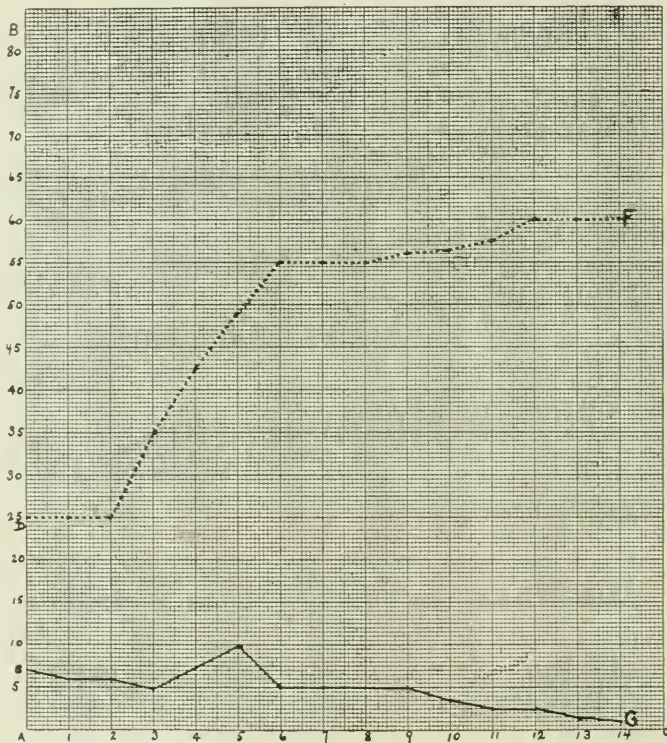


Fig. 1.

AB—Minims required to produce turbidity of test solutions.  
 AC—Number of days following operation.  
 EG—Bile at operation and after operation.  
 DF—Urine at operation and after operation.

normal outflow of bile, a retrograde movement of the bile into the liver undoubtedly occurs. This takes place if stasis alone is present but becomes more marked if actual obstruction also exists. Secondly, absorption of bile into the lymph channels and blood takes place and increased excretion through the kidneys follows. In obstructive jaundice, both the pigment and bile salts appear in the urine, whereas in certain other conditions, notably cirrhosis of the liver, one element may appear in the urine without an appreciable increase of the other. Thus in certain cases one finds an enormous in-



crease in bile salts without the normal coloring matter of the bile, while in others the pigment predominates to the exclusion of bile salts. For this reason, it is obviously wrong to assume that unless bile pigment is present in the urine, there can be no increase in bile salts. Both should be tested for if a clear conception of the changes taking place is desired.

At operation the bile in each case was aseptically collected and tested for its various constituents. A striking feature of this bile was the diminution or absence of bile salts. Numerous specimens gave negative Hay's tests for bile salts and the Pettenkoffer and peptone reactions were likewise negative or very faint. Quantitative determinations of the bile salts present were made by treating the bile with absolute alcohol and then filtering it to remove the mucin or nucleoalbumin present. The filtrate was then tested with definite amounts of peptone and starch solutions and the amount necessary to produce a turbidity of these solutions determined. These were tested against known bile salt solutions and the bile salt content thus determined. The bile salt content of the bile was found to range in cases of cholelithiasis, from  $\frac{1}{64}$  of 1 per cent to  $\frac{1}{2}$  of 1 per cent.

Certain other cases relatively few in number, of catarrhal cholecystitis were seen in which the bile salt content of the bile was found to be low. The bile salt figures in certain of these cases ranged from  $\frac{1}{10}$  -  $\frac{1}{20}$  of 1 per cent. These cases were treated as potential gallstone cases and responded well to cholecystostomy. Other cases have been observed where cholecystectomies were done where the bile salt content of the bile was found to be normal. These were noncalculous cases where the gall bladder was removed for other reasons. It is my belief that in such cases, if the gallbladder presents a healthy appearance, and the bile itself is chemically normal, a cholecystectomy is not indicated.

An organ such as the gall bladder possessing definite functions should not be removed, unless these functions are impaired or destroyed. It should be remembered that in most cases of gall bladder disease we are in reality dealing with hepatic insufficiency. Our problem is therefore, to so treat our cases that the liver again becomes a normally functioning organ. This point will be discussed later in this paper but it is mentioned here because of the present day tendency to remove gall bladders, as if they were functionless organs that the body can easily do without.

The bile obtained in gallstone cases where obstruction was present was usually dark green or black in color, of tarry consistency and relatively rich in mucin. The urine, if examined at this time, gave positive tests for bile salts and contained as large or larger percentages of bile salts than the bile itself.

Following operation, the bile and urine were daily collected and tested out as above described. After drainage of the gall bladder and removal of the stones, the urine tended to show daily decreases in bile salt, and the bile corresponding increases. Daily variations were noted and it was observed that the removal of the obstruction did not lead to immediate return to normal function on the part of the liver. Various grades of hepatic insufficiency



were noted and in two fatal cases the liver did not regain its normal functions even after operation. In these two cases the bile remained practically free of bile salts and greatly diminished as to quantity. The day prior to death only one dram was secreted through the tube in twenty-four hours. The urine in the meantime became scanty in amount, highly concentrated and contained bile salts and pigment in excessive quantities. In these two cases the urine and the bile could be scarcely differentiated by physical appearances.

In the other cases the urine reached normal limits in six to eight days and the bile in ten to fourteen days. About the fourth day after operation and for several days thereafter, an increase in urea occurred in the urine.

#### BILE SALT CONTENT OF URINE BEFORE AND AFTER OPERATION

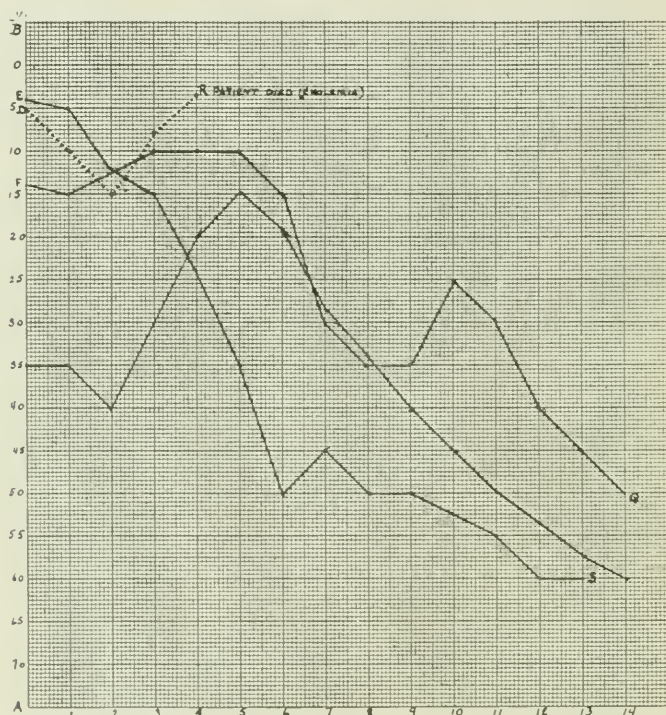


Fig. 2.

AB—Number of minims of urine required to produce turbidity of test solutions.

AC—Number of days following operation.

ES and FQ—Cases that recovered.

DR—Curve seen in fatal cases.

With the increase of urea there seemed to be a decrease in bile salts in the urine.

The accompanying charts graphically represent the changes above described. Both favorable and unfavorable cases are here depicted and the contrast between the two is quite striking. It should be noted also in connection with these observations on the bile that the urinary secretion seems to run almost parallel to the secretion of bile. Suppression of urine is a common complication in fatal cases of cholemia. These processes are not

merely coincidental but rather, the renal injury is dependent on biliary dysfunction with consequent toxemia. The bile, no longer finding access to the biliary channels, and intestine for its excretion, finds its way into the lymphatic and blood streams and the excretion of these toxic substances through the kidney produces a toxic nephritis with suppression of urine. Thus the mode of death is not particularly puzzling or difficult to explain. In reality we are dealing with primary hepatic insufficiency and a secondary nephritis of hepatic or biliary origin. In a subsequent publication the results of certain blood analyses will be reported by Dr. S. Tashiro and myself which further confirm the observations presented in this paper. These find-

BILE SALT CONTENT OF BILE AT OPERATION AND AFTER OPERATION

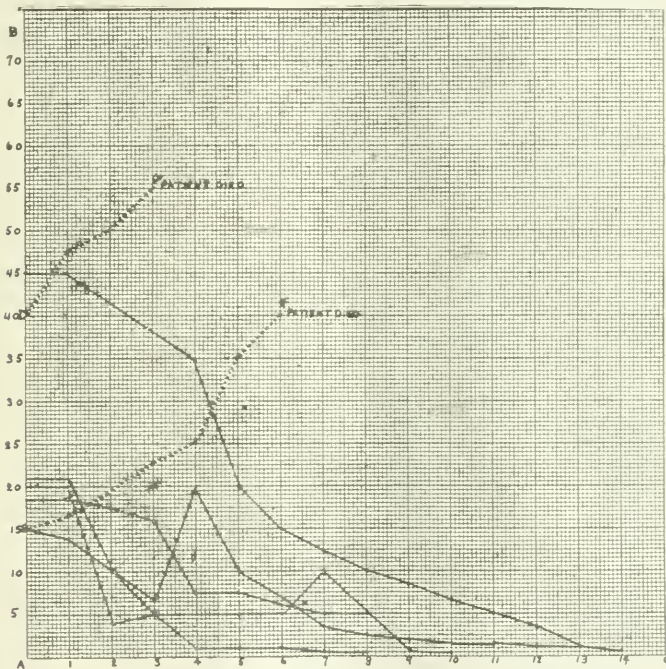


Fig. 3.

AC—Number of days following operation.  
 AB—Minims of bile (alcoholic filtrate) necessary to produce turbidity in test solutions.  
 DG and EF represent curves seen in fatal cases.

ings show that in all such cases as here described, there exists an increased bile salt content of the blood. Together with these findings it should be noted that we are also dealing with a cholesterinemia in all gallstone cases. An increased cholesterin blood content, together with a decrease in the bile salt content of the bile, provides an ideal soil for the formation of gallstones.

The findings above reported are of considerable importance in the elucidation of the formation of gallstones. It is known that the bile salts are the chief elements concerned in holding the cholesterin and lecithin of the bile in solution. Likewise it is known as stated previously, that cholesterin is the chief substance found in most gallstones. The occurrence of an in-

crease in cholesterol in the blood in cases where an increased bile salt content is found is probably in the nature of a protective mechanism. Matthews<sup>11</sup> states that cholesterol neutralizes the toxic action of hemolytic substances such as saponin, glucosides, and bile salts thus protecting the red blood corpuscles of the body from excessive destruction. It accomplishes this purpose by forming a weak molecular union with these substances. The hemolytic action of bile salts on red blood corpuscles is, in all probability, the result of a bile salt-protein chemical interaction and is similar to the effect of bile salts on the pneumococcus and in dissolving the tails of human spermatozoa. The nature of this chemical process will be discussed at length in a subsequent publication dealing with certain bile salt-colloid phenomena.

The occurrence of anemia, often severe in gallstone cases with obstructive jaundice, has been repeatedly observed and is, in all probability, due to excessive blood destruction resulting from the hemolytic action of bile salts.

Another point of interest while discussing cholesterol is that it forms one of the chief constituents found in the deposits on the walls of arteries. The underlying basis for this cholesterol deposit is at the present time conjectural.

In cases of cholelithiasis then, it would seem that an intimate interrelation exists between the cholesterol content of the blood and the bile salt index there found. Further, the relationship between the deposit of the cholesterol in the bile and the bile salt content of this substance is a most important one.

From theoretical grounds, it would appear logical that any condition leading to the removal of the bile salts from the bile should favor the settling out of these substances from the bile.

Bile obtained at operation from gallstone cases, after having been examined chemically, was then placed in test tubes, sealed, and allowed to stand for varying periods of time. It was thought that if the chemical conditions for stone formation were present in such bile, a settling out of cholesterol should occur. Normal bile was also obtained and the different specimens so secured were observed. It was found that those specimens of bile which were deficient in bile salt contained a deposit of cholesterol in the bottom of the tube, often in a very short time. All such specimens showed this change within two to three weeks, while in some it appeared in three to four days. Normal bile or bile rich in bile salts, remained free from cholesterol deposits. This simple experiment was formed with approximately seventy-five specimens of bile obtained at and after operation and the same general changes were observed as described. It could be accurately predicted, after discovering the bile salt content of the specimen, which tubes would show cholesterol deposits and which would not. Curiously enough, in a number of tubes, the cholesterol settled out in concentric layers, alternating with layers of pigment, thus bearing a striking similarity to true gallstones.

As we know that cholesterol is the chief element found in gallstones, a plausible explanation of stone formation here is offered. On this hypothesis



stones need not necessarily be formed in the gall bladder alone, but may be found anywhere in the biliary tract. That they should collect in the gall bladder primarily is to be expected. In all probability they are formed elsewhere as well and are washed into the gall bladder and there retained. The probable steps in the formation of stones are about as follows:

First: There occurs a bile stasis; this is probably most frequently brought about through infection. It is entirely conceivable, however, that stone formation may occur independently of infection. Any factor or factors responsible for bile stasis may be looked upon as predisposing causes.

Second: As a result of the stasis produced, a back-damming into the liver occurs.

Third: Consequent to this change a certain degree of hepatitis and hepatic insufficiency occurs.

Fourth: As a result of this change in physical conditions and impairment of liver function the bile salts are not excreted into the bile as they normally are.

Fifth: If this condition is merely temporary, a condition of "biliousness" probably exists which is relieved by cholagogue cathartics such as calomel and  $\text{MgSO}_4$ .

Sixth: If the condition extends over a considerable period of time the cholesterol of the bile is no longer held in solution and tends to settle out. It tends to collect around some foreign nidus, dragging down a certain amount of pigment and mucin with it. The development of these concretions tends to further increase the stasis present, and, if not relieved by drainage, must inevitably lead to further stone formation. If actual obstruction occurs, a vicious circle is established which can only be corrected by operative intervention. If the process has gone on for a long period, the damage to the liver may be such that only temporary relief will be obtained. This, I believe, accounts for the numerous symptoms which sometimes persist after operation. We must remember that in reality we are dealing with hepatic disease even more than with disease of the gall bladder. If the liver can be restored to a normal state, we have obtained a real cure.

To briefly summarize then, it would seem that in cases of gallstones and obstructive jaundice, a disturbance in bile salt secretion and excretion occurs.

This manifests itself by an increase in bile salts in the urine and blood and a decrease in the bile salt content of the bile.

As a result of the deficiency in the bile salt content of the bile, cholesterol tends to settle out of solution. If the condition is not early remedied, gallstones form the end result of this physico-chemical disturbance. Further the increase in bile salts in the blood leads to toxic changes in other organs, notably the kidneys and heart, and also profound constitutional disturbances of a toxemic nature. In all cases of cholemia, hepatic insufficiency is present.

Following operation, the urine becomes normal usually in six to eight days while the bile reaches normal limits in ten to fourteen days.

Finally, cases of this character should be so treated as to restore the bile, urine and blood to their normal physico-chemical state.



In conclusion I wish to express my very great thanks to Dr. Shiro Tashiro for his unselfish and valuable aid, and to Drs. A. P. Matthews, and Martin H. Fischer, for their many words of counsel and advice.

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## CRESYLECHT VIOLET, A RARE DYE\*

### OBSERVATIONS WHICH SUGGEST THAT IT MAY BE OF VALUE IN PATHOLOGICAL WORK

By B. G. R. WILLIAMS, M.D., PARIS, ILL.

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THIS preliminary report is based upon work begun about 1915 and continued as well as possible during the past seven years with recesses due to difficulty in securing material consequent to the World War. Most of the work was done in my laboratory, but some of it has been carried out at St. Anthony's Hospital (Terre Haute, Indiana), where all excisions are worked up routinely.

Credit for assistance in the work is gladly given to Dr. H. J. Conn, Chairman Committee on Standardization of Stains, National Research Council and to the various American dye manufacturers and jobbers who have furnished materials and who will be mentioned later in this connection.

Cresyl violet (eresylecht violet, not crystal violet) is a rare dye. Apparently it has been used but little or not at all in this country. There is but little in the literature on the subject; and this is foreign. The method of preparing cresyl violet was developed by Bender in 1892. It is a derivative of cresyl blue and is probably (?) prepared from cresyl blue by sulphonization or alkalization to obtain the proper violet shade. Although there are (or have been) a number of stains marketed under the name of cresyl violet, they all have certain properties in common; and cresyl violet is not identical with cresyl blue, as some manufacturers have claimed it to be.

Cresylecht violet is not listed in the main table of Schultz' Farbstofftabellen<sup>1</sup> but it is mentioned in the index of this book where it is referred to presumably as a new or comparatively unimportant dye. In this index three

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shades of cresylecht violet are mentioned, denoted respectively as B, BB and 2RB. It is stated that this dye was manufactured by the Farbwerk Mühlheim of Frankfort a. Main, formerly A. Leonhardt and Company. As will be shown below, it has been difficult to secure under the name, "Cresyl violet or cresylecht violet. Gruebler" any two products with identical properties.

My attention was directed to cresyl violet perhaps ten years ago, but later by the descriptions of Krause<sup>2</sup> who secured beautiful results with tissue sections in normal histology. However the product was mentioned by him as cresyl violet, RB. It was described by him as an oxazin, coming into commerce as a bluish-violet powder, dissolving with the same color production and was claimed by him to give beautiful metachromasia in fresh tissues and formalin fixed tissues. I have never been able to learn of its use in pathological work,\* although cresyl violets have been referred to in connection with normal histological sections. Herxheimer<sup>3</sup> used Kresylecht-violett for staining sections of skin; and Fink<sup>4</sup> used one of these stains for similar work. Also Bielschowsky and Plien<sup>5</sup> employed concentrated cresyl violet, cold for twenty-four hours, differentiated by alcohol, for tigroid substance.

#### WHERE USED IN TISSUE WORK

In my hands the stain has proved of promise in two kinds of pathological work; viz.,

1. In the so-called biopsy, where unfixed tissues are stained for purpose of rapid diagnosis. This method has been used in certain hospitals with a view to securing a diagnosis during an operation, that the surgeon may judge as to the probable extent of the disease or the best method for completing the operation. Here for several years cresyl violet has been giving me better results than I can secure with other stains used for this purpose. Perhaps the staining is not so rapid but there is better metachromasia and the pictures are clearer. Other stains which are being used for this work are thionin, Wilson's stain, Goodpasture's stain, Thibault's stain, Terry's stain, etc., most of them alkaline methylene blue solutions. Roughly the method is to throw several of the sections into a watch glass of the stain (dissolved to concentration or strong solution in cold distilled water and filtered) and removing after a minute or so and examining in water after a brief washing in water. The sections should be fairly thin and should not overlap in the stain. As stated, the method does not seem to be quite so prompt as with the various alkaline methylene blues, but the cells are stained and diagnoses are possible which are hopeless where only tissue schemes are made out. Metachromasia is good, the nuclei (chromatin) taking a deep blue or violet; the cytoplasm, some other shade of blue (depending upon the cell-type); the collagen fibrils taking a deep red; the fibroglia fibrils, pink; fat droplets often a yellow or orange and the erythrocytes appearing as green discs, etc.

2. But a more promising class of work has been that which, for want of a better name, I have called "Diagnostic Surgical Tissue Work" or fairly rapid work with frozen-formalin fixed tissues where a metachromatic stain

\*Since the above was written, I have learned that Warthin has used Kresyl-echt-violett in pathological work, particularly as a specific stain for amyloid and mucin. He has used it for the last ten years. To each gram of the dry stain, he uses 80 c.c. carbolic acid and 20 c.c. alcohol.

TABLE OF RESULTS

	NO. 1197	ORIGINAL BATCH (GRUEBLER)	2ND. SAMPLE (GRUEBLER)
Appearance	Dark blue.	Dark violet.	Dark violet.
Soluble	In water.	In water.	In water.
Stability of Solutions	After 6 weeks is stable in Aq. dest.	Stable in 10% forma- lin but not in water.	Not stable, even in formalin. Blue first lost, leaving a red fluorescent solution.
Color of Solution	Deep blue with a vio- let shade in strong transmitted light.	Deep violet.	Violet.
Color of Wash Waters	Blue.	Deep red and fluor- escent.	Red. Very fluorescent.
Fluorescence	None.	Marked.	Very marked.
Unfixed Tissues	Good results in aque- ous solution.	Good results in forma- lin solution.	Fails to stain in 2 minutes.
Fixed Tissues	Good results in aque- ous solution.	Good results in aque- ous or formalin solu- tion.	In 2 minutes cyto- plasm becomes pink. Eventually the nuclei take a light blue.

## Résumé,—

1. The results with this American cresylecht violet are good. It is possible that this product is similar, identical or better than that described by Krause as cresyl violet RB.
2. The two samples of Gruebler cresylecht violet, are not identical.
3. These dyes seem to consist of two separate parts or properties, a blue and a red. Those which are mainly blue are more stable. They all show metachromasia. Where there is more red the violet is more marked than the blue reaction. Past a certain point the increase of red property gives a very unstable solution and one which does not satisfactorily stain the nuclei.
4. Concerning No. 1197.
  - a. It is much superior to Second Sample (Gruebler).
  - b. It is much more stable than Original Batch (Gruebler).
  - c. It stains more rapidly and more intensely the nuclei than does either other sample.
  - d. It stains materials which are not stained by the Gruebler samples, particularly the fat.
  - e. It does not show the "violet metachromasia" of Original Batch (Gruebler), but it shows a sharp metachromasia and one which is satisfactory for pathological work. By adding to its red properties the "violet metachromasia" might possibly be realized. However this would doubtless be at the expense of its keeping properties in solution and with loss to rapidity of chromatin staining. I would recommend that no changes be made in the dye at present.
5. I would suggest that samples of No. 1197 be tried out by tissue pathologists with unfixed tissues and those fixed by formalin. I am securing best results with frozen sections (not too thick) and to avoid as much as possible overlapping of sections. I am using no celloidin sections. Our staining time varies with the particular tissue, the fixation and the kind of examination desired. It is usually 2—5 minutes. Sections cannot be mounted in balsam. Levulose does not give good mounts. I examine in water after washing in water. Any particular section may be de-stained by alcohol and a special method of staining used. At present I am using a strong or concentrated solution of the dye in cold, distilled water (filtered).

saves time and where many sections can be quickly handled and examined in water under a long rectangular cover glass, often avoiding double-staining, mounting, etc., etc. Or in case special double-staining is wished in any particular section it may be rapidly destained in alcohol and stained as wished for a mount. Roughly I have figured that by this method a dozen sections can be studied in the same time that the more elaborate methods will give one or two. Moreover such a method is of particular value where the diagnosis is fairly easy, where the interested parties are impatient for a diagnosis and where sections should be examined from a number of portions of the neoplasm. It often shows how great the variation of structure in several portions of the specimen, a point missed by the man who glances but one or two sections.

#### EARLY WORK WITH CRESYL VIOLET

After the armistice I found that cresyl violet of foreign make (and this was the only make) was not upon the domestic market, nor could it be secured very easily by importing. I learned this after about thirty-five letters to jobbers, manufacturers, importers, etc.

About twelve months ago, a cresyl violet of American make was put upon the market. This product was tested in my laboratory and found to be insoluble in water. Moreover, as regarded staining properties, it failed to resemble cresyl violet.

Somewhat later I was persuaded that cresyl blue was after all identical with cresyl violet; but investigation proved that it was not. Other substitutes were recommended and some of them tried out; but all failed to give results even approaching those of cresyl violet.

Then Dr. Conn was able to secure for me two samples of cresyl violet which have proved of great value in this work. One of these was some Gruebler cresylecht violet furnished me through the courtesy of the Will Corporation and the other was a sample of American cresylecht violet prepared by the National Aniline & Chemical Company of New York. The results of my investigation of the former showed great variation in the properties of Gruebler cresylecht violets, while study of the latter gave me a material which yielded better results than any of the foreign cresyl violets I have examined. Fortunately I still had on hand some of the Gruebler product secured before America's entry into the World War. Accordingly experiments with the three dyes were set up alongside and the results reported.<sup>6</sup>

In this report, No. 1197 refers to the cresylecht violet supplied by the National Aniline & Chemical Company. By original Batch (Gruebler) is meant the Gruebler stain secured before 1917; and by Second Sample (Gruebler) is meant the Gruebler stain furnished me by the Will Corporation, this last May.

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## A NOTE ON THE DETERMINATION OF UREA IN BLOOD BY THE FOLIN AND WU METHOD. A MODIFIED APPARATUS\*

BY GEORGE G. BOGGS AND W. S. McELROY, PITTSBURGH, PA.

IN THE determination of urea in blood by the Folin and Wu<sup>1</sup> method we have encountered difficulty in the distillation of the ammonia. The acid in the receiver may be drawn back into the distilling tube because of variations in pressure. This does not occur often when the determinations are conducted in a perfectly still room or when the burner is protected from drafts. However, it is not always possible to obtain these favorable conditions in a hospital laboratory or in a student laboratory. During the past two years our students have had considerable difficulty with the method on this account.

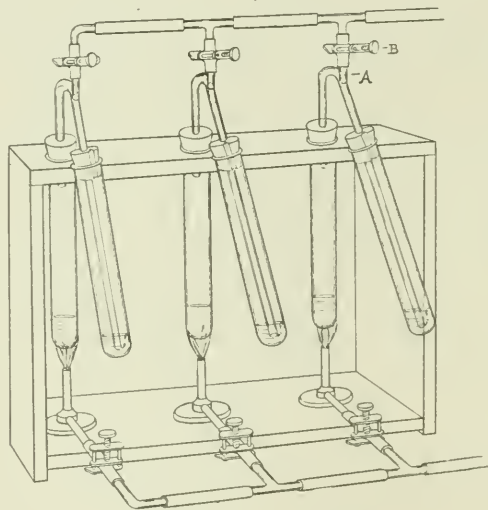


Fig. 1.

Watson and White<sup>2</sup> modified the delivery tube to prevent liquid being carried over as froth into the receiver. This does not overcome the difficulty we have encountered. Excessive frothing is moreover usually due to traces of protein in the filtrate because of incomplete precipitation or to faulty preparation of the enzyme extract.

By passing a slow current of air into the delivery tube so that a slight positive pressure is maintained during the distillation we have found that drawing back of the distillate is prevented. The arrangement employed is shown in Fig. 1. The air is introduced through the side tube A. After

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adding the borax to the contents of the distilling tube the rubber stopper carrying the delivery tube and receiver is firmly inserted into the distilling tube. The air current is started and so regulated by the screw clip B that about one bubble per second issues from the end of the tube in the receiver. The flame is then applied and the distillation conducted in the usual way. A number of determinations may be run simultaneously as shown in the diagram. In the absence of an air line in the laboratory the small volume of air required for a number of analyses may be supplied by air displacement from a large bottle by means of a water siphon.

It is important that the air current enter the connecting tube below the bend as shown in the diagram. If the air current is introduced at or before the bend, difficulty is encountered in driving all of the ammonia over into the receiver. This is probably due to condensation of the vapor in the tube by the entrance of the cool air.

We have found that no ammonia is lost even when a moderately fast air current is used, although this does not promote faster distillation and therefore a very slow current is best. No ammonia is lost if the distillation is prolonged several minutes. If the boiling is too vigorous and prolonged, however, a slight turbidity may appear in the distillate which interferes somewhat with the color comparison after Nesslerization.

We have tried the usual aeration combined with the steam distillation and have obtained good results but the above arrangement is preferable.

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## TECHNICAL PRINCIPLES IN RADIAL TRANSMISSION SPHYGMOGRAPHY\*

BY H. M. KORN, M.D., AND E. J. WARNICK, CLEVELAND, OHIO

THOSE who have worked with radial transmission sphymographs are in position to appreciate the fact that the human forearm, with its varying size, shape, and consistency, offers unsatisfactory support for all such instruments. It is almost impossible to strap the base of the instrument to the

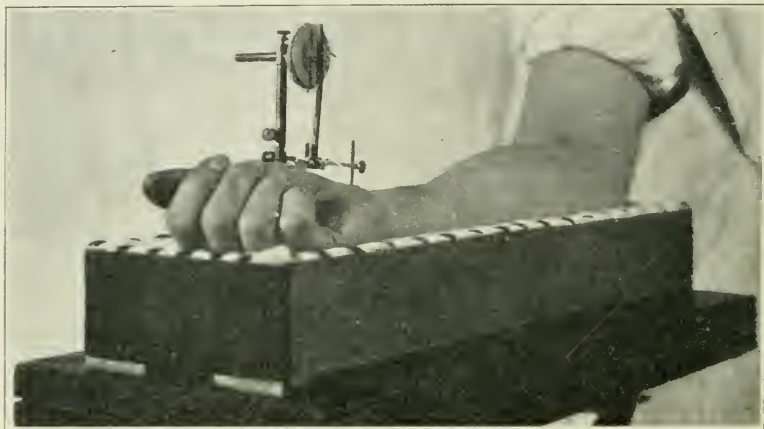


Fig. 1.

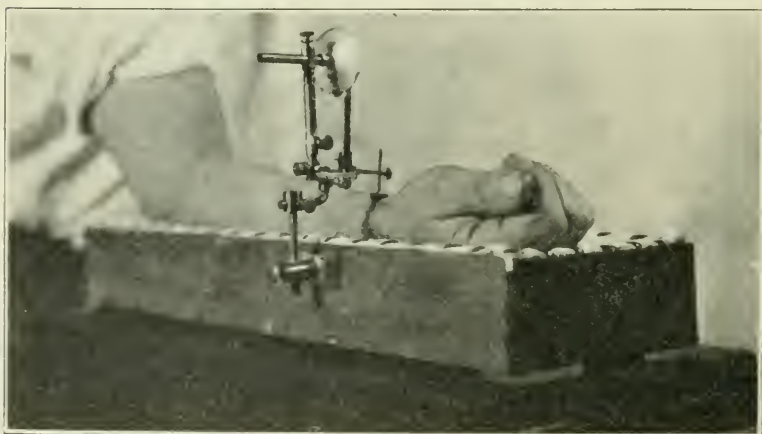


Fig. 2.

arm firmly enough to insure transmission through the sphygmograph lever of all the motion imparted by the radial pulse. Some of this motion is invariably lost through movement of the entire instrument upon the soft tissues of the arm. Furthermore, in order to bring the radial artery into bold relief it is necessary that the forearm be supported in the supine position in such a

\*From the Medical Clinic of Lakeside Hospital.  
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way that the third metacarpal bone forms with the dorsal surface of the wrist an angle of approximately 155 degrees. One cannot expect the subject to maintain this position, for it is essential to his comfort and to good technic that all the muscles of the forearm and hand be maintained in a state of complete relaxation; on the other hand it is difficult to provide suitable extemporaneous support.

To surmount these difficulties we have cast a plaster of paris bed for the dorsum of the forearm, using a moderately large subject as a model. Although this bed is much too large for a thin arm, its essential feature, namely, preservation in a comfortable position of the proper angle between the wrist and third metacarpal bone, is universally applicable. The plaster bed is lined with soft material, and may be additionally padded as required. It is necessary to prepare separate plaster beds for the right and left arms in order that tracings may be made from either radial artery. Absolutely rigid

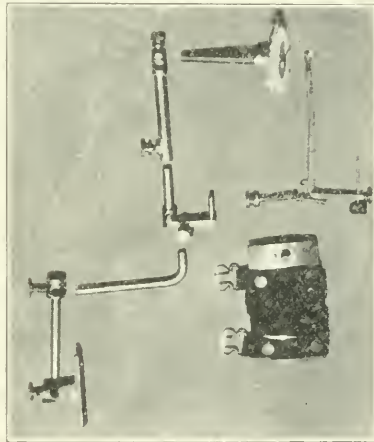


Fig. 3.

support for the sphygmograph is furnished by mounting it on the side of the box which encloses the plaster cast. By this means the wrist is caught firmly between the underlying support and the button resting upon the radial artery, an arrangement which affords ideal technical control. A distinctive feature of the instrument is the provision of ample means for adjustment, so that the optimal application of the button may be easily and quickly secured. The details of the arrangement are shown in the accompanying figures. When the tambour is connected in the usual way to an optical recording capsule the tracings conform in every respect to the requirements for accuracy. The sphygmograph itself is a modified form of that devised by Wiggers and Baker.\* It is interchangeable with the median arm plate, as shown in Fig. 3. Because of the fact that the sphygmograph does not move with the arm, it is necessary, in order to secure an undistorted record, to prevent the slight movements of the arm which normally take place with respiratory excursion of the thorax.

\*Wiggers and Baker: *Am. Jour. Physiol.*, 1922, lix, 454.



## REPORT OF A CASE OF CHRONIC HYDROCYANIC ACID POISONING\*

BY JACOB ROSENBLOOM, M.D., PH.D., PITTSBURGH, PA.

CASES of industrial and chronic cyanogen poisoning are very rare. Weyl<sup>1</sup> states that he could find no case in any of the German factory inspectors' reports for twenty years prior to 1897, nor in some twenty-five volumes of foreign factory inspectors' reports. Rambousek<sup>2</sup> has found practically the same in a search of the modern literature. He reports two cases of cyanogen poisoning. In one case, the symptoms of vomiting, nausea, palpitation and fatigue developed after two years in a man working in the silver electroplating of copper plates where the plates were dipped in a solution of silver cyanide. The other case is one of acute poisoning of industrial nature.

The poisonous action of cyanogen and its compounds depends upon their power of absorption of oxygen from the blood with the result that the venous blood retains the color of arterial blood. This results in a tissue suffocation. At the same time they have an exciting and then a paralyzing effect upon the nervous system.

Most of the cases of chronic poisoning result from inhalation and if large quantities have been inhaled, death occurs almost instantly. In slight cases of poisoning the patient feels a sensation of irritation in the throat, giddiness, sickness, and dyspnea. A form of chronic hydrocyanic acid poisoning has been described in workers manipulating prussic acid and cyanids, with the following symptoms.

The workers complain of oppression of the chest, throat irritation, giddiness, difficulty in breathing, palpitation, hebetude, exhaustion, nausea and vomiting. Karitschoner<sup>3</sup> made some observations on patients who were made to breathe at intervals during many weeks, prussic acid vapor, with the idea that such a treatment would destroy the tubercle bacilli. One-fourth of those treated this way suffered from redness of the pharynx, salivation, headache, nausea, vomiting, slow pulse and albuminuria.

Cases of chronic poisoning have been described in photographers and those engaged in cyaniding gold ores and in galvano plating. Reed<sup>15</sup> has described cases of chronic poisoning from hydrocyanic acid and also chronic poisoning from cyanogen chloride.<sup>16</sup>

On account of the rareness of this condition, I feel the following case should be reported.

Mr. S., chemist, has been working for the past nine months at a chemical problem in which he uses a great deal of hydrocyanic acid. No change was noticed in his health during the first eight months. For the past month, however, he has no energy and vomits every meal, shortly after its ingestion, and he notices that his left eye twitches constantly.

\*Received for publication, October 16, 1922.

A complete physical examination shows normal organs. The pupils are slow in reacting to light and to accommodation, otherwise the neurological examination is negative. The urine is normal. The blood examination showed the following:

Hbg., 70; R.B.C., 3,500,000; W.B.C., 10,000; Diff. Normal.

It can be noted that there is present a moderate secondary anemia.

I feel that his symptoms and the secondary anemia are the earliest symptoms of chronic cyanide poisoning, on account of the fact that after one week's absence from the laboratory, the symptoms disappeared.

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- <sup>16</sup>Reed: Jour. Ind. Hyg., 1920, ii, 140.

# A FATAL CASE OF BACILLUS ACIDI LACTICI MENINGITIS WITH AUTOPSY FINDINGS AND REVIEW OF BACILLUS MUCOSUS INFECTION\*

BY HENRY M. RAY, M.D., PITTSBURGH, PA.

WHILE infection with members of the group of bacillus mucosus capsulatus (Friedlander) with isolation of the organism from the blood is relatively of rare occurrence, investigation of the literature fails to reveal a single case in which the bacillus acidi lactici has been isolated from the spinal fluid. In the case about to be reported, the bacillus acidi lactici was isolated twice each from the blood and spinal fluid during life and also from the brain, spleen, and liver after death.

In addition to the more commonly reported cases of bacillus mucosus pneumonia and septicemia, the organism is mentioned as being the exciting cause in epididymitis, purpura, multiple neuritis, suppurative cholecystitis, liver abscess, otitis media, angina and croup. Two cases of suppurative meningitis attributed to infection with this group are reported by Jassniger, 1901, and Sheib, 1900, the former a Friedländer diplobacillus infection, and the latter an infection with bacillus laetis aerogenes. It is to be noted that in the last two cases, the bacteriologic evidence is inconclusive since the examinations are incomplete and the reactions on the various carbohydrates are not recorded as seems indeed to be the case with many of the other reports of infection with the bacillus mucosus group. The incompleteness of methods employed, especially the failure to plant the organism on all the carbohydrates is largely responsible for the difficulty which apparently still exists in establishing a suitable classification of the members of this group. A practical classification based on the fermentation of certain carbohydrates and first recommended by Perkins has been adopted by many workers and is as follows: (1) The bacillus laetis aerogenes (Escherich) which possesses the most marked power of fermentation, producing acid and large amounts of gas in dextrose, lactose and saccharose broth. This member is probably the one most frequently observed. (2) The bacillus acidi lactici (the bacillus duodenale of Forde) possesses fermentation powers in a lesser degree and produces acid and gas in dextrose and lactose broth, but fails to attack saccharose, inosite, raffinose, zylose or inulin. The amount of gas production is much less than that produced by the bacillus laetis aerogenes. (3) The bacillus pneumoniae (Friedlander) ferments dextrose and saccharose to acid with a small quantity of gas production, but fails to ferment lactose. The discrepancies reported by bacteriologists in the reactions of this group to the

\*From the Laboratory of Clinical Pathology, South Side Hospital, Pittsburgh, Pa.  
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carbohydrates are in all likelihood due to the fact that in many instances impure carbohydrates are used and in addition, the failure to recognize the fact that organisms occasionally lose their original fermentative powers as a result of changes in environment or other unfavorable conditions.

Jasniger's case occurred in a 16-year-old apprentice who suddenly became ill with severe chills, passing into a state of stupor and developing within a few days rigidity of the neck, hyperesthesia, irregular pupils and a definite Kernig with incontinence of urine and feces. Temperature rose above 39° C., with irregular remissions. Death occurred on the 7th day and the autopsy revealed a purulent cerebrospinal meningitis. Direct smears showed many intra- and extracellular capsulated bacilli with rounded ends. Cultures on agar, broth and gelatin produced a similar organism which was nonmotile and gram negative. A rabbit survived an intraperitoneal inoculation with 1/2 c.c. of broth culture while a mouse succumbed within 12 hours. No fermentation reactions on the carbohydrates are recorded and the author concludes that the organism was the *diplobacillus pneumoniae* of Friedlander.

Scheib's case was that of an 8-day-old girl, one of a pair of twins. The child died without showing any signs or symptoms of organic disease and the clinical diagnosis was "debilitas vitae." Postmortem examination, which was performed 8 days after death revealed a suppurative cerebral meningitis with distention of the tympanic cavities with pus. There were no other significant changes. The author gives a brief morphological description and after stating that the usual media were inoculated, he concludes that "taken in all, the evidence presented proves that the bacterium *lactis aerogenes* was present in the corpse." Here again no fermentation reactions on the various carbohydrates were attempted and considering the fact that there were no symptoms or signs of organic disease and that the postmortem was performed 8 days after death, it is highly improbable that there was an antemortem *bacillus aerogenes lactis* infection at all.

The case occurring in this hospital was that of a white American 55 years old, admitted to the medical service of Dr. C. L. Palmer, February 15, 1922, with the statement that 5 weeks previously he began to experience pain in the chest, cough, difficulty in breathing, severe headache and impaired appetite. The family history offered nothing of importance and the previous history stated that there had been the usual diseases of childhood and about 10 years ago, a fractured jaw.

The physical examination showed a well nourished middle-aged man apparently in a stuporous state and responding slowly to questions. Pupils were contracted and equal and there was a distinct arcus senilis. Examination of the chest revealed a few scattered moist râles with impairment of resonance over both bases. Heart sounds were distant, free from adventitious sounds and there was no apparent enlargement of the precordial area. The abdomen was scaphoid and tympanitic. The reflexes could not be elicited. The temperature on admission was 102° F. and varied between 99° and 104° with irregular evening remissions. The pulse was full and soft and varied between 92 and 134. Respirations at first averaged 24 to the minute,



increasing during the last two weeks to 42. Blood pressure was 196 systolic and 100 diastolic.

The course of the disease while in the hospital was characterized by marked confusion with severe headache alternating with periods of dullness. There developed definite rigidity of the neck with slight left-sided hemiplegia. Roentgenologic examination revealed marked density of the upper two-thirds of the right lung with intervening aerated spaces suggesting bronchopneumonia. The blood count showed hemoglobin 65 per cent, red blood cells 3,600,000, white cells 18,200 and 76 per cent polynuclear neutrophils. Urinalysis revealed a trace of albumin and an occasional hyaline cast. The widal was negative in all dilutions. Blood Wassermann was negative to both crude and cholesterinized antigens and the blood chemical determinations were nonprotein nitrogen 51 mgs., urea 32, creatinin 0.9, uric acid 2.1, and sugar 120.

Blood cultures on various media taken February 25, and March 1, revealed in both instances numerous colonies of gram negative bacilli showing the interesting characteristics soon to be described and corresponding to the bacillus acidilactici. A blood culture repeated 20 days later remained sterile after 72 hours. The spinal fluid, on two occasions (February 27 and March 7) yielded upon culture the identical organism. Both specimens of spinal fluid were a distinct canary yellow, globulin two-plus, and reduced Fehling's solution. Cell count, February 27, showed 108 with 92 per cent polynuclear leucocytes and March 7, 80 with 91 per cent polys. The colloidal gold curve of both fluids was distinctly of the meningitis type. The direct smears from the centrifugalized sediment revealed many gram negative bacilli which exhibited distinct capsules when stained by the Hiss, Rosenow and Nicolle methods.

The cultures recovered twice from the blood and spinal fluid revealed an organism which was a gram negative, nonmotile, nonspore bearing bacillus exhibiting a distinct capsule. The colonies on the blood plates appeared as slightly transparent, bluish white, rounded bodies with irregular outline and showing under the high power a delicate network of furrows. The bacilli averaged 1 to  $2\mu$  in length by  $.5$  to  $.7\mu$  in diameter and the ends were somewhat rounded. There were no irregularities in staining. Transplants into carbohydrates produced acid and gas in dextrose, maltose, mannite, arabinose, rhamnose, sorbitol, dulcitol, mannose, lactose and dextrin. It did not produce acid or gas in saccharose, inositol, raffinose, xylose and inulin. It produced acid on litmus milk and gave a positive indol reaction. Gelatin was not liquefied and after four days the extension of the growth along the line of the stab presented the typical nail-like appearance. Growth was luxuriant in practically all media and on agar it formed a thick white viscous layer. Milk was coagulated and smears from the milk culture exhibited the capsules most beautifully. Broth was diffusely cloudy after 24 hours and on the surface a viscous pellicle formed, making a ring around the tube; on further incubation the pellicle fell to the bottom but the medium remained cloudy.

A guinea pig inoculated intraperitoneally with  $\frac{1}{2}$  c.c. of a 24-hour broth

culture died within 4 days and showed upon examination a sanguino-fibrinous peritonitis with bronehopneumonia. The organism was recovered from practically every organ in the body.

Intravenous inoculation of 1 c.c. of a 24-hour broth culture into the marginal vessel of a rabbit resulted in death within 48 hours. The organism was demonstrable in all the viscera and in the brain. The morbid anatomy corresponded to that of any hemorrhagic septicemia.

The patient died after a 28-day stay in the hospital and the autopsy was performed 1 hour after death with the following findings:

*Anatomic Diagnosis.*—General. The bacillus acidi lactici bacteriemia. *Head.*—Acute and subacute fibrino-purulent meningitis; marked pial edema; multiple disseminated petechial hemorrhages of the cerebrum, cerebellum, pons and medulla; sclerosis of cerebral arteries. *Mediastinum.*—Acute and subacute mediastinitis with hemorrhagic infiltration; subacute adhesive pleuropericarditis; acute and subacute serofibrinous pleuritis. *Lungs.*—Unresolved lobular pneumonia; pulmonary congestion and edema; anthracosis. *Heart.*—Hydropericardium; fatty infiltration of the epicardium and myocardium; multiple petechial epicardial hemorrhages; cardiac dilatation with relative insufficiency of the valve orifices. *Peritoneal Cavity.*—Encapsulated retroperitoneal abscess. *Liver.*—Fatty cirrhosis. *Spleen.*—Acute splenic tumor with multiple intracapsular hemorrhages. *Pancreas.*—Sclerosis. *Adrenals.*—Cloudy swelling with congestion. *Kidneys.*—Congestion. *Prostate.*—Involution with sclerosis.

*Résumé of Protocol.* The body is that of a well developed and obese white male of good musculature and nutrition, weighing 190 pounds and measuring 68 inches in length. Right pupil is irregular and dilated and the left contracted. Skin of the chest, abdomen, lower extremities and in fact the mucosa of the lips are studded with innumerable pin-point to pea-sized petechiae which persist in spite of pressure. Abdomen is distended and tympanitic. The ankles pit on pressure.

In the right lower abdomen, entirely walled off, and retroperitoneal is an abscess cavity the size of an adult fist and bounded by the lumbar vertebrae, the ileo-psoas muscle and the posterior abdominal wall. The cavity contains about an ounce of blood-tinged purulent fluid which infiltrates the muscle; no connection with the vertebrae or cord can be demonstrated.

Pleural cavities contain bloody fluid and present posteriorly some dense adhesions. The visceral pleura shows an adherent stringy fibrinous exudate. Right lower lobe is distinctly nodular and the cut surface drips a bloody fluid which in the region of the nodules is quite free from air bubbles. Cut surface of the nodules is a deep red, finely granular and projects slightly. The other lobes show an intense grade of edema but no naked eye evidence of consolidation.

The pericardial cavity contains about 3 ounces of clear yellow fluid. Heart is enlarged, particularly the right side, and the apex is somewhat blunted. Epicardium shows a marked excess of fat and a large number of pin-point to pea-sized petechiae. Myocardium is hypertrophied and the cut surface shows a number of grayish white to yellow flecks. Right chambers are dilated and contain fluid and clotted blood. Valve orifices are relatively dilated and the endocardium is free from vegetations.

Liver is somewhat reduced in size and presents a smooth glistening capsule through which the parenchyma appears as a distinctly yellowish substance. The consistence is increased and the cut surface shows a definite increase in interlobular connective tissue. A distinctly greasy fluid can be scraped from it.

The spleen is increased one and one-half the normal size. The capsule is tense and studded with innumerable pin-head to pea-sized hemorrhages. The substance is firm and the cut surface presents an extremely friable and readily disintegrated pulp in which the malpighian follicles are quite conspicuous from swelling.

The pancreas is normal in size and shape. The consistence is greatly increased, the substance cuts with resistance and the cut surface presents a diffuse, grayish, finely granular or even smooth tissue in which the normal lobulation is not apparent to the naked eye.

Adrenals appear to be somewhat enlarged and softened. The section reveals a greatly widened and blood infiltrated medulla.

The kidneys, bladder, testicles and gastrointestinal tract show no significant naked eye changes.

Removal of the calvarium reveals a most marked grade of subpial edema, the pia in places bulging from underlying accumulations of yellowish fluid. In other places there are seen through the membranes collections of greenish pus which is rather thick, fibrinous and adherent. The purulent meningeal infiltration is almost exclusively confined to the cerebral vertex. A large quantity of thin purulent fluid is present at the base. The vessels remain patent and the walls are atheromatous. Section of the brain reveals innumerable irregularly scattered punctate hemorrhages. Ventricles are dilated and distended with a milky fluid.

Cultures from the heart blood, from the retroperitoneal abscess and from the liver, spleen and brain reveal an organism morphologically and culturally identical with that isolated from the blood and spinal fluid during life and corresponding to the *bacillus acidi lactici*.

#### SUMMARY

A case of *bacillus acidi lactici* meningitis and septicemia is reported with isolation of the organism from the blood and spinal fluid before death and from the viscera and brain after death. There is no record in the literature of another case of *bacillus acidi lactici* meningitis.

The patient presented all the classical signs of meningitis and the pathogenicity of the organism was demonstrated by its virulence for rabbits and guinea pigs.

Thanks are due Dr. G. R. Lacy of the Singer Memorial Research Laboratory for his kind interest in independently confirming the bacteriological findings.

NOTE: Dr. Ray has prepared a full reference to the literature of this subject which cannot be published on account of its length.—*Editor*.

# LABORATORY METHODS

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## THE RELATIVE VALUE OF SOME OF THE COMMONLY USED METHODS FOR THE DETECTION OF OCCULT BLOOD IN THE STOOL\*

BY HOBART A. REIMANN, M.D., BUFFALO, N. Y.

FROM the conflicting reports of various authors on the results obtained in testing for occult blood by some of the more commonly used methods at our disposal, it appeared advisable to test the relative value of these methods on a series of normal and pathological cases, the results of which are submitted below.

### EXPERIMENT

Patients were selected from the wards, who had no evident lesion in the gastrointestinal tract and special care was taken to exclude pyorrhea, bleeding gums or hemorrhoids. Stools were tested for the presence of occult blood and the patients placed on a meat-free, chlorophyll-free diet, regardless of result, for the remainder of the experiment. After three or four days, all stools gave negative reactions.

Approximately 30 c.c. of blood was withdrawn from the median basilic vein, oxalated and placed in the ice box.

Each patient was then given an increasing dose of his own blood every day. The stools examined, were marked with green corn or carmine in order to tell approximately whether the quantity of blood given the day before was in the sample of feces tested. Agar was occasionally used to insure a daily stool. Only formed stools were used.

The blood was given at the noon meal usually in a small quantity of cocoa or cocoa meringue. The total quantity of the mixture was small so that all would be eaten. When the doses of blood were greater than 3 c.c., one-half the dose was given at dinner and the other half at supper.

*Technic.*—The whole stool was thoroughly stirred and one gram placed in a test tube and stirred with 5 c.c. of water and 2 c.c. glacial acetic acid. Then 2½ c.c. of ether was added and the mixture was thoroughly shaken. Finally 2½ c.c. more of ether was added and the tube inverted 10-15 times slowly.

*Benzidine test.*—Equal parts (5 c.c.) of saturated solution of benzidine (10 per cent) and hydrogen peroxide were mixed and overlaid with the ethereal extract.

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\*From the Laboratory of Clinical Pathology, Department of Laboratories, Buffalo General Hospital. Received for publication, Oct. 12, 1922.



TABLE I

## RESULTS

CASE AND AMT'S OF BLOOD	BENZIDINE 10%	GUAIAIC 1-60	BENZIDINE GREGERSON TECH.	GUAIACONIC ACID	SPECTROSCOPIC
J. S. Neuras- thenia					
0 c.c.	neg.	neg.			
1 c.c.	very faintly pos.	neg.			
2 c.c.	very faintly pos.	neg.			
4 c.c.	strongly pos.	pos.			
Repeat					
0 c.c.	neg.	neg.			
4 c.c.	pos.	faintly pos.			
H. A. R.					
0 c.c.	very faintly pos.	neg.			
1 c.c.	neg.	neg.			
1½ c.c.	faintly pos.	neg.			
2 c.c.	strongly pos.	very faintly pos.			
2½ c.c.	strongly pos.	faintly pos.			
Repeat					
0 c.c.	neg.	neg.	neg.	neg.	
3 c.c.	pos.	neg.	pos. in 3 minutes	neg.	
4 c.c.	strongly pos.	faintly pos.	strongly pos.	neg.	
5 c.c.	strongly pos.	faintly pos.	pos.	very faintly pos.	
9 c.c.	strongly pos.	pos.	strongly pos.	very faintly pos.	
T. R. Nephritis					
0 c.c.	very faintly pos.	neg.			
1 c.c.	faintly pos.	neg.			
2 c.c.	pos.	neg.			
3 c.c.	faintly pos.	neg.			
4 c.c.	faintly pos.	neg.			
A. W. Br. Asthma					
0 c.c.	neg.	neg.	neg.	neg.	
1 c.c.	pos.	neg.	neg.	neg.	
1½ c.c.	neg.	neg.	neg.	neg.	
2 c.c.	strongly pos.	neg.	faintly pos.	neg.	
3 c.c.	neg.	neg.	neg.	neg.	
5 c.c.	strongly pos.	neg.	pos.	neg.	
6 c.c.	pos.	neg.	pos.	neg.	
F. K. Arterio- sclerosis					
0 c.c.	neg.	neg.	neg.	neg.	
1 c.c.	neg.	neg.	neg.	neg.	
1½ c.c.	pos.	neg.	faintly pos.	neg.	
2 c.c.	pos.	neg.	pos.	neg.	
3 c.c.	strongly pos.	neg.	pos.	neg.	
4 c.c.	strongly pos.	very faintly pos.	strongly pos.	neg.	
J. Y. Multiple neuritis					
0 c.c.	neg.	neg.	neg.	neg.	
2 c.c.		neg.	pos.	neg.	
3 c.c.		pos.	pos.	faintly pos.	
6 c.c.		strongly pos.	strongly pos.	very faintly pos.	

TABLE I—CONT'D.

## RESULTS

CASE AND AMT'S OF BLOOD	BENZIDINE 10%	GUAIAIC 1-60	BENZIDINE GREGERSON TECH.	GUAIACONIC ACID	SPECTROSCOPIC
F. B. Sciatica					
0 c.c.	neg.	neg.	neg.	neg.	
2 c.c.		neg.	faintly pos.	neg.	
3 c.c.		faintly pos.	pos.	neg.	
4 c.c.		neg.	faintly pos.	neg.	
8 c.c.		pos.	strongly pos.	very faintly pos.	

## PATHOLOGICAL CASES

C. G. Carcinoma stomach		pos.	strongly pos.	pos.	
J. K. Cholemic hemorrhage		very strongly positive	very strongly positive	pos.	Neutral methemoglobin bands
S. S. Carcinoma stomach		very strongly positive	very strongly positive	pos.	Faint neutral methemoglobin bands
N. A. Duodenal ulcer		pos.	strongly pos.	pos.	Faint acid hematin bands
J. S. Carcinoma stomach		pos.	strongly pos.	pos.	Faint acid hematin bands

The following are some quantitative results obtained with the various reactions when blood was added to originally negative stools:

PROPORTION OF BLOOD TO STOOL	GUAIAIC	BENZIDINE GREGERSON TECH.	GUAIACONIC ACID	SPECTROSCOPIC
1-60	strongly pos.	strongly pos.	pos.	Acid haematin bands
1-84	strongly pos.	strongly pos.	pos.	neg.
1-120	pos.	strongly pos.	pos.	neg.
1-225	pos.	strongly pos.	faintly pos.	neg.
1-600	pos.	pos. in 1 minute	doubtful	
1-1200	faintly pos.	pos. in 3 minutes	doubtful	
1-2250	neg.	pos. in 4 minutes	neg.	
1-6000	neg.	pos. in 6 minutes	neg.	
1-12000	neg.	pos. in 8 minutes	neg.	

*Guaiac test.*—Gum guaiac, .08 gm., was dissolved in 5 c.c. alcohol (1-60 sol.) equal parts (5 c.c.) of this solution and hydrogen peroxide were overlaid with the ethereal extract.

*Gregerson's test.*—Benzidine, .02 gm., and .1 gm. barium peroxide were dissolved in 5 c.c. of 50 per cent glacial acetic acid and the solution overlaid with the ethereal extract.

*Guiaconic acid.*—Two c.c. of the ethereal extract were treated with a solution of the preparation of guaiac, prepared according to the method of Lyle and Curtman,<sup>1</sup> and one to five drops of 30 per cent perhydrol was added drop by drop.

*Spectroscopic.*—The ethereal extract was examined by a direct vision spectroscope.

#### DISCUSSION OF RESULTS

With patients on a full unrestricted diet, practically every stool was positive to both benzidine and guaiac. The writer also included himself in the experiments and after a heavy meat diet gradually placed himself on a meat-free, chlorophyll-free diet. The stool from the heavy meat diet produced a positive benzidine and a faintly positive guaiac reaction. The stools became less and less positive to benzidine and negative to guaiac until the fourth day after the last meat was eaten, when both benzidine and guaiac reactions were negative. The result corresponded exactly to that of Dewis' experiment.<sup>2</sup>

The practice of introducing a patient's own blood into his stomach should correspond more or less to the behavior of blood from a bleeding ulcer or cancer, and would seem to be of much greater use in estimating the value of a test for occult blood than is the addition of blood to a stool after the latter has passed through the alimentary tract, and has gone through all the processes of digestion.

Theoretically, a patient receiving 1 c.c. of blood one day passes a stool containing the remains of 1 c.c. of blood the day following, and if 2 c.c. is given that day, he will pass the remains of 2 c.c. the next day and so on. But there is no way of telling how much of the stool has passed through or how much is retained from the day before, so that the results can never be exact. Furthermore the rate of digestion, the action of individual enzymes, the rate of bowel movement and the nature of the food eaten certainly all have a modifying effect on different individuals or in the same individual at different times. However, in the case of a marked stool in a normal individual with a normal evacuation, one may be reasonably certain of obtaining most of the hemoglobin ingested or rather the substance to which the reaction is due. It is evident that the stool must be thoroughly mixed to obtain a representative sample.

When preparing the ethereal extract we have found that a clear ether is always obtained if part of the ether is thoroughly shaken with the stool emulsion and the remainder is merely mixed by inverting the test tube ten to fifteen times. We found, in accordance with the finding of Dewis, that it is unnecessary to boil the mixture before extracting. Removal of fat is also unnecessary.

The original benzidine test, employing a saturated solution of benzidine gives a dark-colored underlying solution, so that faint reactions of green color are easily missed. We found the guaiac test to be on the whole, quite satisfactory and reliable and it is a valuable check to the benzidine reaction.

The technic proposed by Gregerson is the most reliable of the methods used and gives the most clean-cut reaction.

The use of guaiaconic acid prepared according to the formula of Lyle and Curtman<sup>1</sup> gave us positive results only with amounts of blood over 3 c.c. or in a dilution of 1-200 as shown in the control table. besides being

quite troublesome to make, but has remained constant in strength (in solution) for ten months after its first trial. In order to overcome the usual objection to guaiac, namely the lack of uniformity of gums, we purchased a considerable amount of powdered gum and thoroughly mixed it. This is used in exact quantities is reliable after knowing the strength of reaction to known amounts of blood.

The spectroscope in our hands was only of value when the blood in the stool was present in proportions of 1-60 and in confirming the presence of blood in large quantities, in which case it is the most definite of all tests.

It was found in accordance with most authors<sup>4, 1</sup> that the factors of paramount importance in accurate tests are the use of constant reagents and proportions and clean glassware. However it is only necessary to clean the test tubes used with scouring soap and hot water. The precise laborious chemical methods of Gattner and Schlesinger<sup>5</sup> were found superfluous. Another factor which must of course not be neglected in interpreting the test is as emphasized by many investigators, namely, the absolute necessity of keeping a patient on a meat- and chlorophyll-free diet for at least three days previous to the test.<sup>3, 6, 8, 9</sup>

As regards the delicacy of the reaction, we could not, as Cowie states, detect an intestinal hemorrhage of 1 c.c. by the guaiac method. We found that at least 2 c.c. is necessary.

As to the spectroscopic examination our findings also differ materially from those of Boas.<sup>10</sup> According to the latter, the spectroscopic findings are identical with that of the Gregerson technic and 2 c.c. of blood introduced into the stomach can be detected. We have obtained positive benzidine reactions (Gregerson technic) with 2 c.c. of blood also but we found that over 6 c.c. are necessary before the hemochromogen stripe can be obtained in the spectroscope. Our principal difficulty was the density of the color of the ethereal extract which tended to obscure the absorption bands, and removal of the color without removal of the hematin is not possible. Von Domarus<sup>11</sup> states that the hemoglobin derivative bands are best seen when the dilution of the substance lies between 1-50 and 1-200.

Grünwald finds that the benzidine test is positive with 1 part of blood in 150,000, guaiac with 1 to 2000 and the spectroscope 1 in 5000.

Our results show the benzidine test (Gregerson technic) positive with 1 part of blood to 12,000, guaiac with 1 in 1200, spectroscope 1 part to 60.

Lyle and Curtman<sup>1</sup> find that their preparation is positive to a 1 to 10,000 solution of blood. In our hands it becomes definitely positive only when the dilution of blood is 1-225, greater dilutions being doubtful, on account of the color of the ethereal extract, or entirely negative.

It seemed that the best and most convenient method to date is the Gregerson technic, checked by the 1 to 60 solution of guaiac. For convenience we have powder papers made containing .02 gm. benzidine and .1 gm. barium peroxide and powder papers containing .08 gm. of powdered gum guaiac. When dissolved in 50 per cent glacial acetic acid and 95 per cent alcohol respectively, these make solutions of proper and constant strength, and are al-



ways ready to use. We agree with Wolf<sup>6</sup> that benzidine (Gregerson technic) is a test delicate enough but not too delicate and guaiac a test not too coarse to ascertain the usual amounts of occult blood found in the stool.

It seems perfectly possible that, with the use of constant chemicals giving reactions to quantities of blood known beforehand, the quantity of blood passed in feces could be approximately determined. Thus the carefully controlled benzidine and guaiac reactions could be made quantitative tests, making it possible to estimate the rate of hemorrhage. Other investigators have recently brought forth the same view.<sup>12</sup>

If the objection of the hypersensitiveness of the benzidine test is eliminated by the routine control with the guaiac test we possess in it a very reliable test for occult blood. Certainly more useful than the spectroscopic test so strongly recommended by Snapper<sup>13</sup> and others.<sup>10</sup>

#### CONCLUSIONS

1. A positive reaction for occult blood in the stool with any of the methods in use is, in accordance with most authors, valueless, when the patient has not been put on a meat- and chlorophyll-free diet and has not had thorough evacuations for at least three days prior to the test.

2. The most reliable and convenient test for occult blood in the stool is the Gregerson benzidine technic.<sup>6, 10</sup> The use of the guaiac test in conjunction with it is recommended in order to gain some idea of the quantity of blood if the amount present be between 1 and 5 c.c.

3. The spectroscope is not recommended for the detection of traces of blood.

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## THE QUANTITATIVE ESTIMATION OF IODINE IN URINE\*

By H. LARUE MARSH, M.S., CHICAGO, ILL.

IT FREQUENTLY becomes desirable to determine iodine eliminated during iodine therapy in a twenty-four-hour specimen of urine. None of the methods described in the literature are wholly satisfactory. Since some of the iodine is eliminated in organic combination, it is obviously necessary to precede an analysis with a fusion of an evaporated sample of urine.

The method described below is recommended not only as one embodying the very necessary fusion, but also as one well adapted to the usual apparatus, reagents, and experience of the clinical chemist. It consists of: first, the determination of the total halogens in the residue from a fused sample of urine by the Volhard-Arnold<sup>1</sup> volumetric method; second, the determination of the chlorides (Volhard-Arnold) in another fused sample from which the iodine has been removed by the classical method of Gooch.<sup>2</sup> The difference between the total halogens and the chlorides represents the iodine present in the sample of urine.

### PROCEDURE

Fifty c.c. samples of the urine are evaporated to dryness in nickel or silver dishes with 35 c.c. of a solution of a fusion mixture containing 40 gm. of sodium hydroxide and 200 gm. of sodium nitrate per liter. This evaporation can be best done in a gas oven. When dry, the residues are fused till free from carbon, cooled, and dissolved in not more than 100 c.c. of distilled water. During fusion the dishes should be covered with watch glasses to avoid possible losses by spattering.

The total halogens are determined in one sample. The solution is quantitatively transferred to a 250 c.c. volumetric flask and 110 c.c. of tenth normal silver nitrate are added from a burette, after which 15 c.c. of concentrated chloride-free nitric acid are added. The addition of the acid *after* the silver nitrate avoids possible losses of iodine. The solution is now shaken till effervescence has ceased, made up to the mark, mixed and filtered through a dry filter into a dry container. One hundred c.c. of the clear filtrate are transferred to a 250 c.c. Erlenmeyer flask, 1 c.c. of a saturated solution of chloride-free ferric alum is added, and the excess of silver nitrate present is titrated against a tenth normal potassium sulphocyanate solution till the first permanent coloration is produced.

The chlorides are determined in a second sample, the fusion of which having been dissolved, is transferred to a two-liter Erlenmeyer flask and

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diluted to a volume of approximately one liter with distilled water. To this is added 3 c.c. of concentrated chloride-free sulphuric acid and 10 c.c. of dilute nitrous acid, chloride free (or 0.5 to 1.0 gram of chloride-free sodium nitrite). The solution is boiled until all the iodine has been expelled and the solution has become colorless. In all cases the solution should be boiled for at least thirty minutes. One hundred ten c.c. of tenth normal silver nitrate are now added from a burette. The addition of the silver at this point is necessary to prevent loss of chlorides during subsequent concentration. The solution is boiled down to a volume of 200 c.c. or less. (Bumping may be avoided and evaporation hastened by passing a current of air through the solution.) When concentrated sufficiently, the solution is cooled to room temperature and transferred to a 250 c.c. volumetric flask and made up to the mark with distilled water. From this point the procedure is exactly the same as described for the first sample.

The iodides present are calculated from the differences between the total halogens and the chlorides. The method used on samples of urine containing known quantities of sodium iodide gave results (average of duplicate determinations) as shown in Table I:

TABLE I

SAMPLE OF URINE NUMBER	GRAMS OF NaI PER 100 C.C. OF URINE PRESENT	GRAMS OF NaI PER 100 C.C. OF URINE FOUND
1	0.1098	0.1089
2	0.1098	0.1086
3	0.1098	0.1089

In conclusion it may be stated that the above described method is simple in technic and gives dependable and accurate results.

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## A SIMPLE METHOD FOR CALCULATING THE BASAL METABOLIC RATE\*

BY RUSSELL L. HADEN, M.A., M.D., KANSAS CITY, KANSAS

BY common consent the results of the basal metabolism determination are expressed in terms of calories of heat produced per square meter of surface area per hour. It is usually assumed that the respiratory quotient is 0.82, hence 4.825 is the number of calories taken as resulting from the consumption of a liter of oxygen. In the closed circuit type of apparatus such as the Benedict the metabolic rate is determined from the amount of oxygen consumed over a certain period of time. For many workers the calculation

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The factor at any temperature and pressure represents the following:  
BC is the barometer correction.

TABLE I

TC x BC x 60 x 4.825  
1000

where TC is the temperature correction and

P. (mm.)	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°
700	0.249	0.248	0.247	0.246	0.245	0.244	0.242	0.241	0.240	0.239	0.238	0.236	0.235	0.234	0.233	0.232
705	0.251	0.250	0.248	0.247	0.246	0.245	0.244	0.243	0.242	0.240	0.239	0.238	0.236	0.235	0.234	0.233
710	0.253	0.252	0.250	0.249	0.248	0.247	0.246	0.245	0.244	0.242	0.241	0.240	0.238	0.237	0.236	0.235
715	0.254	0.253	0.252	0.251	0.250	0.249	0.247	0.246	0.245	0.244	0.243	0.241	0.240	0.239	0.238	0.237
720	0.256	0.255	0.254	0.253	0.252	0.251	0.249	0.248	0.247	0.246	0.245	0.243	0.242	0.241	0.240	0.239
725	0.258	0.257	0.256	0.255	0.254	0.253	0.251	0.250	0.249	0.247	0.246	0.245	0.244	0.243	0.241	0.240
730	0.260	0.259	0.257	0.256	0.255	0.254	0.252	0.251	0.250	0.249	0.248	0.246	0.245	0.244	0.243	0.242
735	0.262	0.261	0.259	0.258	0.257	0.256	0.253	0.251	0.250	0.249	0.248	0.246	0.245	0.244	0.243	0.242
740	0.264	0.263	0.261	0.260	0.259	0.258	0.256	0.255	0.254	0.251	0.250	0.248	0.247	0.246	0.244	0.243
745	0.265	0.264	0.263	0.262	0.261	0.259	0.257	0.256	0.255	0.254	0.253	0.251	0.250	0.249	0.248	0.247
750	0.267	0.266	0.265	0.263	0.261	0.259	0.257	0.256	0.255	0.254	0.253	0.251	0.250	0.249	0.248	0.247
755	0.268	0.267	0.266	0.265	0.264	0.263	0.261	0.260	0.259	0.258	0.257	0.255	0.254	0.253	0.251	0.250
760	0.270	0.269	0.268	0.267	0.266	0.264	0.263	0.262	0.261	0.259	0.258	0.257	0.255	0.254	0.253	0.252
765	0.272	0.271	0.270	0.268	0.267	0.266	0.264	0.263	0.261	0.260	0.258	0.257	0.255	0.254	0.253	0.252
770	0.274	0.273	0.272	0.270	0.269	0.268	0.267	0.265	0.264	0.263	0.262	0.260	0.259	0.258	0.257	0.256
775	0.276	0.275	0.273	0.272	0.271	0.270	0.267	0.266	0.264	0.263	0.262	0.260	0.259	0.258	0.257	0.256
780	0.278	0.277	0.275	0.274	0.273	0.272	0.270	0.269	0.268	0.267	0.265	0.264	0.262	0.261	0.259	0.259



from the data thus obtained is more laborious and time consuming than the actual running of the test.

The calculation requires first of all the reduction of the oxygen as measured to 0° C. and 760 mm. barometric pressure. This corrected gas volume is then multiplied by 60 to give the oxygen consumption per hour and next by the factor to obtain the total calories of heat produced. The resulting figure is finally divided by the surface area as determined from the height and weight by the formula or graph of DuBois<sup>1</sup> (Fig. 1). This final result is the basal metabolic rate. It has only to be compared with the DuBois standards for sex and age<sup>2</sup> (Table II) to determine whether it is plus, minus or normal.

McCaskey<sup>3</sup> gives a table for making the temperature and pressure cor-

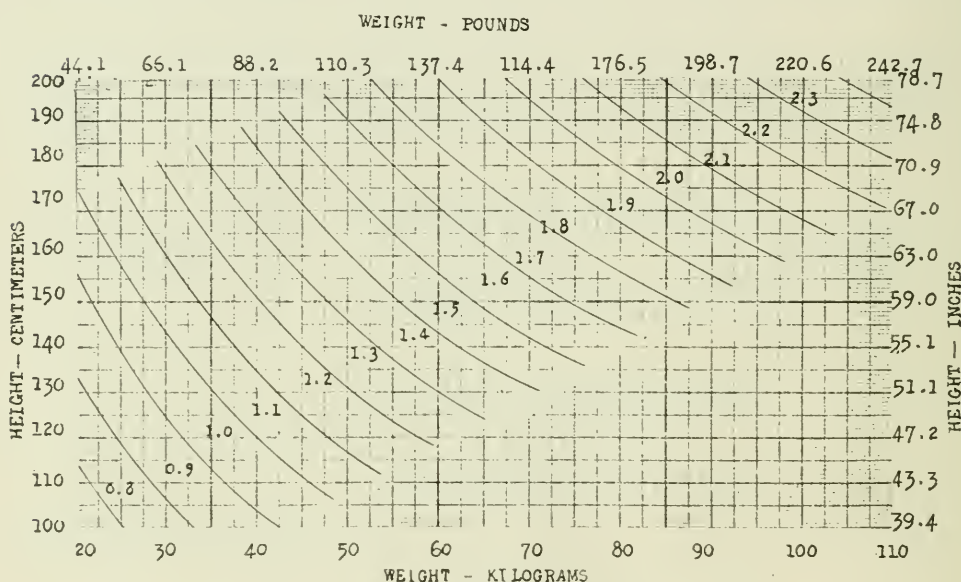


Fig. 1.—Chart for determining surface area of man in square meters from the weight in kilograms (Wt.) and height in centimeters (Ht.) according to the formula:  $\text{Area (Sq. Cm.)} = \text{Wt.}^{0.425} \times \text{Ht.}^{0.725} \times 71.84$ . (DuBois). The equivalents of the centimeters in inches and the kilograms in pounds are given.

rections. The table is correct only when soda lime which will absorb moisture is employed in the test. Newcomer<sup>4</sup> has suggested several aids in calculation based on logarithmic tables. These for the ordinary person are confusing rather than helpful. Similarly Smith<sup>5</sup> has worked out a graph for calculating the metabolic rate in terms of cubic centimeters per minute instead of calories per hour.

For the past two years I have used a table which simplifies the calculation of the basal metabolic rate a great deal. Formerly the table was based on the assumption that the air in the apparatus is practically moisture free, and vapor tension can be ignored. This is true only when caking soda lime or an additional bottle of calcium chloride is employed. At the present time almost every one is using the noncaking soda lime as perfected by Wilson.

TABLE II  
STANDARDS OF METABOLISM (Du Bois)

AGE	MALE (Calories per hour per sq. m.)	FEMALE (Calories per hour per sq. m.)
14-16	46.0	43.0
16-18	43.0	40.0
18-20	41.0	38.0
20-30	39.5	37.0
30-40	39.5	36.5
40-50	38.5	36.0
50-60	37.5	35.0
60-70	36.5	34.0
70-80	35.5	33.0

This absorbs very little moisture so the air in the apparatus is about 80 per cent saturated. I have recalculated my table (Table I) on the basis of the table given by Wilson<sup>6</sup> for the temperature and pressure correction of 80 per cent saturated air.

In making the calculation the total amount of oxygen consumed during the period of the test is divided by the number of minutes the test is run to give the oxygen consumption per minute. The factor corresponding to the temperature and nearest barometer reading is found in Table I. This factor represents the following:

$$\frac{TC \times BC \times 60 \times 4.825}{1000}$$

where TC is the correction for temperature and BC is the correction for barometric pressure.

The product of this factor by the oxygen consumed per minute in cubic centimeters represents the total heat production. This has only to be divided by the surface area as determined from the DuBois chart and compared with the normal for that age and sex.

The calculation is best illustrated by an example. The patient is a woman age 17. In a ten-minute period 2610 c.c. of oxygen are consumed, or 261 c.c. per minute. The temperature is 27° C. and the pressure is 745 mm. The factor corresponding in the table is 0.250. The total heat production is:  $261 \times 0.250 = 65.2$  calories. The weight of the patient is 132 pounds, and the height 5 feet 5 inches, so the surface area is 1.63 square meters. The basal metabolic rate is:  $\frac{65.2}{1.63} = 40$  calories per square meter per hour. This is just normal for a girl of 17 as will be seen from Table II.

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## AN APPARATUS FOR CLEANING BLOOD COUNTING PIPETTES\*

BY RUSSELL L. HADEN, M.A., M.D., KANSAS CITY, KANSAS

FOR accurate blood counting it is absolutely necessary that the pipettes used be carefully cleaned and dried. The process is a tiresome and time-consuming one if many pipettes are to be cleaned. Most laboratory workers make use of some type of suction apparatus usually employing a piece of rubber tubing attached to a water suction pump. Gray† describes such a method by which one or more pipettes may be cleaned and dried. He sug-

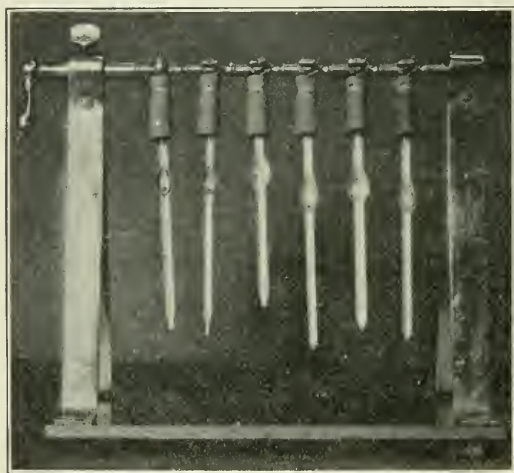


Fig. 1.

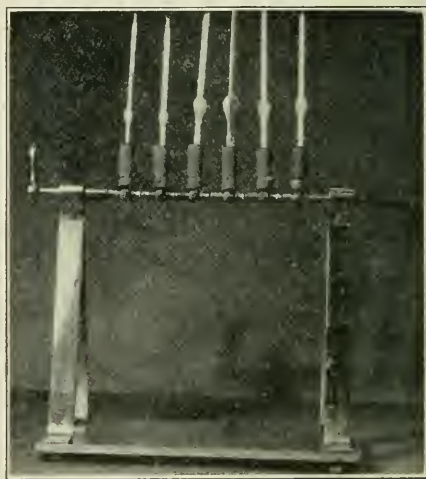


Fig. 2.

Fig. 1.—The apparatus is shown in position for filling the pipettes with water, alcohol or ether. The rubber tube at the right connects with the suction pump.

Fig. 2.—Apparatus in position for emptying pipettes.

gests that the pipettes be dried by gently heating while suction is applied.

I have for some time been using an apparatus with which one or more pipettes can be cleaned and dried thoroughly and rapidly. The device is illustrated in Fig. 1. It is made by soldering the desired number of three-way stopcocks together. Six is enough as the cleaning can be done so quickly more are seldom necessary. The best type of stopcock is the small one made to fit the tip of a Luer syringe. The stopcock must be so made that the air can be drawn straight through and in by the side inlet at the same time.

One end of the soldered stopcocks is closed with a plug or with solder, the other is connected by a piece of rubber tubing to a water suction pump. The apparatus should be supported on a metal frame set on a heavy metal

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†Gray, II., Jour. Am. Med. Assn., 1921, lxxxvi, 1826.

base. A handle is attached at the closed end so the pipettes can be tipped up for emptying. To each side inlet tube a short piece of pressure tubing is attached in the lumen of which the pipette is placed.

After all the pipettes to be cleaned have been placed in position the suction is turned on and water is run through each pipette. The handle is then turned so that the pipettes are vertical with the tips in the air (Fig. 2). The pipettes may be dried by flaming gently with Bunsen burner as suggested by Gray or by running through in succession alcohol and ether.

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### FORMALDEHYDE TEST IN LUES\*

BY D. A. JOHNSTON, M.D., CINCINNATI, OHIO

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IN the search for a chemical test for lues, Gate and Papacosta of Italy noticed that addition of liquor formaldehyde to blood serum caused it to gel in the presence of syphilis, and otherwise not.

Blood is drawn as for a Wassermann. At the end of twenty-four hours the serum is decanted from the test tube into another clean but not sterilized tube. A drop of ordinary commercial liquor formaldehyde is added, and the tube plugged with cotton. The serum and formaldehyde are allowed to remain at ordinary room temperature for twenty-four hours. At the end of this period observation is made as to the condition of the serum. Coagulated serum is a positive result: fluid serum is negative.

Echer of Cleveland reports 37.09 per cent of plus formal tests agreed with plus of Wassermann, 44 or 8.8 per cent of formal plus were of plus type, and of these 13, or 29.54 per cent were Wassermann plus.

Kingsbury, London, says that less than one-half of serums giving plus Wassermann, show coagulation with formalin and nearly 10 per cent of nonsyphilitic serums give a plus result.

A. Terzani, Florence, Italy, obtained conflicting findings in 226 tests with this method.

A. Bouttiau, Paris, concludes from 1500 applications of the test that the reaction is strictly specific for syphilis.

My series of 100 cases is composed of 100 consecutive male admissions to the hospital, all being insane patients. The results are not only interesting for what they disclose as to the value of the formaldehyde test but also for what they show relative to the incidence of lues in general among the insane commitments.

In our Wassermann tests we employ the ice box fixation method, as well as the water-bath, using both the plain and cholesterinized antigen.

In 27 per cent the Wassermann was plus; in 23 per cent the formaldehyde test was plus; in 12 per cent the two tests correspond; in 11 per cent the for-

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maldehyde was plus with negative Wassermann findings; in 15 per cent Wassermann was positive with negative formaldehyde test.

From this will be seen the tendency to induce confusion, and the fact that the test is of no diagnostic value because of the failure to react clinically and serologically in clear cut cases of lues, and also because of the occurrence of plus reactions in absence of disease.

The following table summarizes the psychotic findings:

PSYCHOSES REPRESENTED IN THE 100 CASES

Paresis	26	Arteriopathic	3
Constitutional Psychopathic Inferior	14	Alcoholic	3
Dementia Praecox	12	Involution Melancholia	2
Senile Dementia	10	Psychoses with somatic diseases	2
Manic Depressive	9	Narcotic	2
Paranoic Conditions	6	Epileptic	1
Psychoses with mental deficiency	6	Huntington's Chorea	1
Psychoneuroses	3		
		Total	100

## A SUGGESTION FOR A PRACTICAL SPIROMETER VALVE\*

BY G. A. BROUGH, A.B., B.S., CHICAGO, ILL.

TO accurately determine tidal air with the spirometer an efficient valve is necessary. Any valve which requires considerable energy for operation—which presents obstruction—increases the normal breathing movements. Likewise a valve which permits escape of air during slight movements or does not close promptly is very unsatisfactory. To eliminate some of the unsatisfactory features of the flap valve (Chauveau), and the liquid valve (Müller), a membrane valve was constructed.

The construction can be seen from the accompanying diagram which is almost self-explanatory. The only adjustments necessary are the contacts of the glass outlet (a) with rubber dam (b), and at the inlet (c) and (d). The rubber dam covers the end of a glass cylinder held in place by three pieces of rubber tubing. The glass outlets and inlets are held by rubber stoppers which have been cut to fit.

The valve is reliable, always opening and closing, and is entirely free from the disadvantage of sticking open or closed; the air resistance to the breathing movements has been reduced to a minimum; the valve operates during the most feeble respiratory movements and does not leak, however slight such movements may be; the operation is instantaneous with the initiation of any movement of air; no operative adjustments necessary and no

\*From the Laboratory of Pharmacology and Therapeutics, University of Illinois, College of Medicine, Chicago, Illinois.

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moving mechanical parts to wear after valve is assembled; moisture was found to be beneficial to the action; and from the standpoint of convenience in ex-

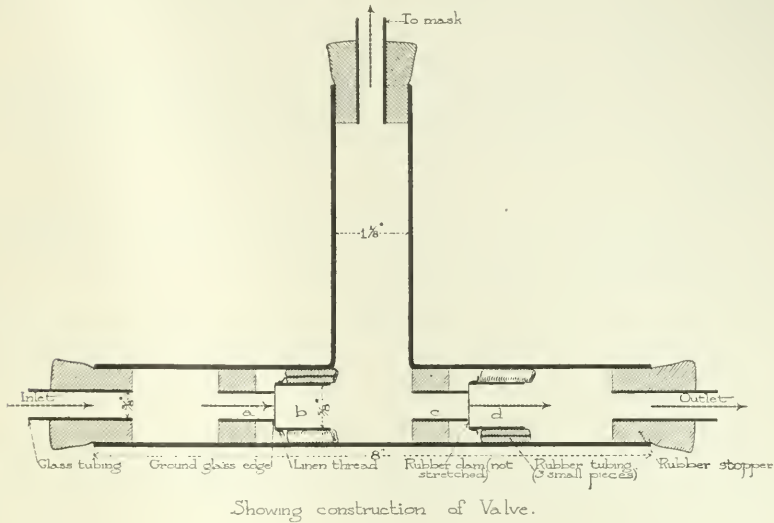


Fig. 1.

perimentation it may be noted that the valve will operate in any position.

The valve units may be assembled in short tubes and connected by means of a T-tube. This permits ready change of direction and rapid replacement.

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## CONCERNING THE SPECIFICITY OF CHOLESTERINIZED ANTIGENS IN THE SEROLOGIC DIAGNOSIS OF SYPHILIS: THIRD COMMUNICATION\*

BY ROBERT A. KILDUFFE, A.M., M.D., PITTSBURGH, PA.

IN previous communications concerned with this subject,<sup>1, 2</sup> the following facts were emphasized:

1. That cholesterolized antigens seemed destined to form a permanent part of the complement-fixation technics devised for the serologic diagnosis of syphilis.
2. That, if such antigens were prone to give nonspecific and false fixations in a large proportion of cases, the factor of error thus introduced forbade their use as the sole antigen in any technic utilized for the serologic diagnosis of syphilis.
3. That complement-fixation technics relying entirely upon cholesterolized

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antigens for the detection of syphilitic reagin were, nevertheless, rather widely used.

4. That, therefore, the evolution of a concerted opinion as to their reliability was indicated.

The only means whereby such an opinion can be materialized seems to be by the collection and analysis of data concerned with the establishment of the reliability or nonreliability of cholesterin-plus reactions as a serologic indication of syphilis.

In the previous articles an attempt was made in a small way to gather data concerned with this problem, and the purpose of the present communication is to present further data of like character.

As a result of an investigation into the merits or demerits of a new complement-fixation technic recently proposed for standard adoption by Kolmer<sup>3</sup>, the following conclusions, among others, were deemed warranted:

1. That the Kolmer modification possessed great delicacy.
2. That insofar as the series showed, false positives did not occur.
3. That while, because of factors inherent in and concerned with the formation or production of syphilitic reagin, the method was not infallible, occasionally failing to detect its presence, the occurrence of a positive reaction by this technic constituted very strong presumptive evidence of the presence of syphilitic reagin almost invariably, when evidence was obtainable, corroborated by clinical and other findings.

Under these circumstances it seemed of interest to note the results obtained with cholesterin-plus serums when tested by what seems to be a most delicate and reliable technic.

It may be argued that as the antigen devised by Kolmer<sup>4</sup> is a polytropic cholesterinized extract to which has been added acetone-insoluble lipoids, the comparison of the two methods is of little value as affecting the status of cholesterinized antigens. The new antigen, however, from the standpoint of delicacy and specificity is so far in advance of any with which the writer is familiar as to be practically different and distinct from the ordinary plain alcoholic extract cholesterinized and the comparison of the results obtained by the two methods seems, therefore, justifiable.

In the course of this investigation 1014 serums were examined by two methods the details of which have been previously published.

There were in the series 91 serums reacting only to the cholesterinized extract (alcoholic extract of human heart with 0.4 per cent cholesterin). In 64 of these or 70 per cent a positive reaction was also obtained with the Kolmer modification. Of these 64 cases 43 were known to be syphilitic, many being cases under treatment; in 10 cases no data was obtainable; and 11 were prostitutes in whom the possibility of syphilis cannot be overlooked.

There remain 27 serums with which negative reactions were obtained by the Kolmer modification.

It has been noted above that the analysis of the results obtained with the Kolmer method lead to the conclusion that a positive reaction by this method

constitutes very strong presumptive evidence of the presence of syphilitic reagin.

The same degree of reliance, however, cannot be placed in a single negative reaction by this—or any other—technic as indicative of the absence of syphilitic reagin, so that it is possible that syphilitic reagin might be truly indicated by the cholesterolin-plus reaction and the same serum still negative—*on a single examination*—by a more delicate and specific method.

This fact of the inconstant production and presence of syphilitic reagin; the fact that it is not produced in mathematical proportion in various stages of the disease, and that unaccountable and inexplicable variations in the reactions obtained occur, constitute one of the inherent factors of error in complement-fixation reactions upon an appreciation of which is based the agreement upon the necessity for more than one negative test before the absence of syphilitic reagin can be reasonably maintained.

The fact that a negative Kolmer reaction was obtained in these 27 serums is not sufficient evidence, therefore, upon which to assume their nonspecific reaction with the cholesterolin antigen in the other technic.

Owing to circumstances beyond control no historical or clinical data for or against the presence of syphilis in these cases is at hand and the question must be left in doubt.

The high percentage of agreement in the other 64 cases, however, coupled with the fact that these serologic findings were corroborated in a large proportion of these cases, seems sufficient evidence, in conjunction with what has been previously presented, upon which to reiterate the claim that the cholesterolin-plus reaction cannot be justifiably looked upon as without significance; and to again urge the collection and analysis of a sufficiently extensive series of such reactions to place the question upon a sound and agreed footing.

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## EDITORIALS

### *Bismuth as a Substitute for Arsphenamine*

THE use of bismuth in the treatment of syphilis was developed in France chiefly through the efforts of Sazerae and Levaditi, who recognized the value of the earlier uncompleted work of Santon and Robert of the Pasteur Institute. The preliminary observations made by Santon and Robert were published in *Annales de L'Institut Pasteur* in 1912. No further experimental work was performed until after the war when in 1921, Sazerae and Levaditi published their preliminary observations. In January 1922, a symposium on the use of bismuth in the treatment of syphilis appeared in the *Annales de L'Institut Pasteur*. Between January and October 1922, twenty-three articles on the use of bismuth in syphilis have been recorded in the quarterly cumulative index, coming from the following eight countries: Argentina, Denmark, Italy, Brazil, Uruguay, Germany and Switzerland. During the same period the treatment was not discussed in the American medical press.

Santon and Robert observed that infection of hens with *spirocheta gallinarum* may be prevented by the preliminary intramuscular injection of a bis-

muth emetin compound. Treatment of the disease with bismuth compounds gave promising but not conclusive results. Sazerac and Levaditi carrying this work forward, experimented chiefly with sodium-potassium tartrobismuthate. They found that this compound could be administered to rabbits in a dosage of from 50 to 60 mg. per kgm. body weight without deleterious effect, when given subcutaneously or intramuscularly. A dose of 200 mg. resulted in death within two to four days. Intravenously the drug is highly toxic, 5 mgs. causing death at the end of a week. The drug was first administered in watery solution and later suspended in oil, with more satisfactory results. The lessened toxicity from intramuscular injection is apparently due to temporary fixation of bismuth in the tissues, with resulting delayed absorption. On dissection, an extensive white deposit is found in the connective and muscular tissue. Müller of Mainz has corroborated this finding and has shown in roentgenograms taken on successive days, that the bismuth casts a shadow of decreasing intensity for three days after intramuscular injection.

The toxicity of an oily suspension is less than that of an aqueous solution of tartrobismuthate administered in the same manner. Oral administration of the drug had no effect upon the progress of syphilis in the experimental animal. Rectal administration caused a temporary but not permanent disappearance of the syphilitic lesions. This method of treatment is being studied more thoroughly. Analogous results were obtained by inunctions. The authors point out the possible advantages to be gained in prophylaxis from the use of bismuth ointment.

Sazerac and Levaditi have utilized various other combinations of bismuth, such as the ammoniacal citrate, the soluble lactate, the subgallate and oxyiodogallate, but found each to be either less active or more toxic than the tartrobismuthate of sodium and potassium.

In the treatment of human syphilis these authors report very promising results, the drug is unusually stable and of low toxicity when given subcutaneously or intramuscularly. Its intravenous administration is absolutely contraindicated. They recommend the use of a suspension in oil.

Fournier and Guénot have collaborated with Sazerac and Levaditi in the clinical use of sodium-potassium-tartrobismuthate. They give the drug intramuscularly in a 10 per cent suspension in olive oil and emphasize the importance of depositing it into the muscles rather than subcutaneously. The latter method is decidedly more painful while the inconvenience from intramuscular injection is no greater than that observed after intramuscular mercury. A total of two or three grams of bismuth should be given during the first month of treatment. Two or three injections of 0.2 grams are given daily, after which the patient receives 0.3 grams twice weekly throughout the month. After this period, treatment may be continued with weekly injections of 0.2 to 0.3 grams or the patient may be allowed one month rest, after which the regular course is repeated. The only necessary precaution for the patient to observe is a careful hygiene of the mouth.

Under this treatment spirochetes disappear from the primary sore after the first to the third injection. The chancre becomes completely healed within

five to twenty-five days, usually within two weeks. The Wassermann reaction, if negative at the initiation of treatment, usually remains so. If positive, the strength of the reaction falls as satisfactorily or perhaps more so than after the use of arsphenamine.

Bismuth is particularly useful in the contagious stages and according to the authors, causes more rapid and complete disappearance of the contagious lesions, than does arsphenamine. In secondary syphilis the cutaneous lesions disappear as a rule within a week. The strength of the Wassermann reaction is very favorably influenced, becoming practically negative within two or three months as a rule, but varying considerably as with other forms of treatment. Tertiary lesions improve with almost equal rapidity. This is particularly true of gummata and tertiary skin and mucous membrane manifestations. The authors report no conclusive observations on visceral or nervous syphilis.

No untoward effects from the use of bismuth have been reported other than a tendency toward stomatitis similar to but usually not as severe as that following the use of mercury. A marginal pigmentation usually appears in the gums, analogous to the familiar lead line. The stomatitis is usually a fusospirillary infection similar to that of Vincent's angina. It is satisfactorily treated by the methods customarily employed in Vincent's angina and may even be rapidly cured by the local application of the tartrobismuthate in powder form. Bismuth appears in the saliva but in an altered form, probably combined with sulphur, in which it has lost its spirillicidal properties.

The drug has been found present in the blood and the cerebrospinal fluid, and it is eliminated in the saliva, the bile, feces, sweat, milk and in the urine. It appears in the urine within from eighteen to twenty hours after injection and persists for from twenty to thirty days after discontinuance of the course of treatment.

Marie and Foucade have used the drug in general paralysis without distinct improvement. The results are analogous to those following neoarsphenamine and mercury. The spinal fluid Wassermann reaction remains uninfluenced although the blood Wassermann is improved. The cases were well advanced when treatment was instituted.

Hugo Müller, director of the skin and venereal clinic at Mainz, has corroborated the work of the French observers and reports equally satisfactory results. He reports unusually good results in the treatment of secondary syphilis accompanied by hypertrophic papules and rupeal syphilis, which are ordinarily rather highly resistant to the usual methods of treatment. He points out, also, that the drug is particularly useful in those cases where the spirochete has become arsenic resistant. Such cases clear up rapidly under bismuth treatment. He reports for example one patient who developed severe mercury poisoning from 1 gram of gray ointment, intense iodism after taking 0.5 grams of potassium iodid, and an acute dermatitis lasting two months following a dose of 1 gram of neosalvarsan.

Grenet, Drouin and Richon have produced an aromatic bismuth compound of the probable formula  $C_{12}H_7O_6Na_3(COOBi(OH)^2)^2$ , which may be injected intravenously without toxic effect.

The immediate results from the injection of bismuth compounds in syphilis appear from the published reports to be as good as or better than those obtained with mercury and arsenic compounds. In any case, the drug will be valuable for alternative use with the latter, in those cases where the infecting parasite has apparently acquired a degree of tolerance to the drug. It will be several years before the end results from bismuth treatment can be comprehensively tabulated. In fact it has not been until recently that we have come to realize the significance of the late unsatisfactory accidents from standard treatment with mercury and arsphenamine.

The discovery of the *treponema pallida* by Schaudinn and Hoffmann, the cultivation of the spirocheta by Noguchi, the application of the Bordet-Gengou phenomenon to the serologic diagnosis of syphilis by Wassermann and the preparation of salvarsan by Ehrlich have concentrated our attention on attempts to rid the body of the spirochete and to obtain a serologic cure, and have diverted our thoughts from the patient as an individual, so that in many instances we have lost sight of the importance of clinical cure. This is often not obtained until long after the Wassermann reaction has become negative.

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—W. T. V.

### *A New Liver Function Test*

IN an editorial discussion of liver function in the June number of this Journal, it was suggested that functional tests carried out on blood alone would eliminate some of the confusion arising from coincident renal or biliary tract abnormality. This method of procedure should theoretically give more accurate information concerning certain phases of liver function. Since that time Rosenthal has published three reports on a new method for testing liver function with phenoltetrachlorophthalein. In the first he describes experimental results, devoting particular attention to the rationale and procedure of the test; in the second he reports his experiments on animals; and in the third, he summarizes the clinical results observed in man.

Five mg. of phenoltetrachlorophthalein per kgm. body weight is injected intravenously and the rapidity of the disappearance of the dye from the blood serum is determined. Normally within fifteen minutes, but 2 to 6 per cent is recovered, practically all being in the serum and none absorbed into the red blood cells. After from forty to sixty minutes the dye is no longer present in the circulation. In hepatic derangements high percentages may be found in the plasma many hours after injection. The standard for comparison is easily prepared by adding stated amounts of the dye to the patient's serum obtained before injection of the test substance. Sera obtained at the end of fifteen-minute and longer intervals are compared with the standard.



Rosenthal finds the highest grades of retention in acute hepatitis, in atrophic cirrhosis and in advanced carcinoma of the liver. In jaundice the concentration of the dye was not found to parallel the intensity of the icterus. Normally none appears in the urine. In hepatic disease, from a trace to 4 per cent was usually present but the percentage gave no indication of the degree of retention in the blood, and in some cases showing blood retention no dye appeared in the urine.

Following widespread parenchymal damage from experimental chloroform or phosphorus poisoning, highest degrees of retention in the blood stream were observed. When repair had taken place curves approaching normal were again obtained.

Following ligation of the common duct the dye was removed from the blood stream within practically the normal interval except that a trace remained present throughout the experiment. Rosenthal observes that in jaundice due to mechanical obstruction, the curves differ widely from those found in jaundice associated with extensive degeneration of the hepatic cells.

Rosenthal has also developed a "ring test" which he finds more sensitive than gross mixing of serum and reagent.

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—W. T. V.

#### *Position of Doctors in the Province of Nikolaev, Ukraine*

THROUGH the courtesy of Dr. Haigh, a member of the Health Committee of the League of Nations and health expert attached to the Nansen Organization, we have the following information concerning the position of doctors in the above-mentioned province. He states that the assistance given to these doctors has so far been negligible and that no organization for their relief exists in the province. A few of these doctors have friends abroad and occasionally receive parcels of foodstuffs. Their salaries are paid after long delays. Their tragic position continues to grow worse. During the winter of 1921-1922 some supplies were officially distributed among them, but even this relief has now come to an end. Many doctors are able to subsist only by the sale of their furniture or other possessions. Even those who have the best practices are in need of clothing. Those who live in remote districts have to rely on the help of such peasants as have managed to escape ruin. Without the official ration, which consists of a pound of maize distributed among a portion of the staff in the hospitals, life would be impossible in these establishments. In September, 1922, the hospital doctor received a salary of twenty-seven million rubles, equivalent to about \$5. Since that time the value of money has decreased. Nurses receive about twenty million rubles and other members of the staff still less. Many doctors and nurses have died at their posts.

—V. C. V.

### *The Present Endemicity of Yellow Fever*

BEFORE his death General Gorgas dreamed that he might see and might participate in the complete eradication of yellow fever from the earth. This was a reasonable ambition, since he had accomplished so much in this direction. When Gorgas began his work in Cuba a large percentage of foreigners coming to that Island was attacked by this disease and many succumbed. At that time yellow fever was widely distributed through the West Indies and throughout Central and South America. The work of Gorgas, both in Cuba and on the Canal Zone, imitated ably and successfully by others, led to the rapid contraction of the epidemic area of this disease. Today yellow fever is known to exist only in Mexico, in a small area in Brazil, and to some extent on the west coast of Africa. That this disease can be entirely eradicated in a given locality has been demonstrated beyond a doubt. There has been no trace of this disease in Havana since the work of Gorgas and his companions. Rio de Janeiro, likewise, has remained free from every trace of it for years. There has been no case of yellow fever in Guayaquil since 1919, and, as we have stated, the possibility of banishing this disease wholly from the face of the earth seems justified. Mexico is one of the countries in which it still lingers, though only in one or two localities where a case now and then is recognized. Through the courtesy of Dr. Vasconcelos, of the Mexican Health Department, we have been kept informed concerning yellow fever in our sister republic. During the month of September, 1922, there were reported in Mexico ten cases, with seven deaths. During the month of October, nine cases, with seven deaths. This is certainly gratifying when we recall that in 1920 there were in Mexico 505 cases of recognized yellow fever, with 249 deaths, and in 1921 there were 115 cases, with three deaths.

Epidemiologists, at least some of them, say that while infectious diseases have been stamped out in certain localities, even permanently, there is no evidence that any such disease, up to the present time, has been wholly and completely banished from the earth. We know of no fact which contradicts this statement but we hope that within a few years yellow fever may be swept from the earth.

—V. C. V.

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### *The Preparation of Staining Solutions*

IN the text books dealing with microscopy and with bacteriology, as well as in the Journal literature dealing with these subjects, there are two general types of stain formulae. In one type of formula so much dry stain in weight per given volume of solution is called for, in the other type so many cubic centimeters of a saturated solution of the stain. It will be readily seen that the same staining formula could be prepared by both methods provided the solubility of the dry stain and its actual dye content were known.

If all batches of dry stain contained the same amount of actual dye either sort of formula would be perfectly satisfactory.

Unfortunately, however, different lots of any stain vary greatly in the amount of inert material they contain. This is true even in regard to different batches of Grüber stains and it is especially true now when there are so many different brands of stains on the market. An investigation of certain methylene blues, for example, showed a Grüber sample examined to contain 57 per cent of actual dye, a Merek sample 55 per cent, while five different American samples varied from 69 per cent to 88 per cent. It seems in general desirable that the color strength should be as high as reasonably possible, and it must be particularly pointed out that the sample containing 88 per cent dye proved one of the two very best in a long series of methylene blues examined. It is obvious, however, that a staining formula calling for so many grams of Grüber methylene blue will be more concentrated if made up with one of these American stains. Considering how these stains vary in actual dye strength, much more nearly constant formulae can be obtained provided they are prepared on the basis of a definite volume of a saturated solution. Although there are undoubtedly some differences in the amount of actual dye that goes into solution in the case of different brands of stains, nevertheless, the results are much more constant in this case than when the formulae are prepared on the basis of weight of dry dye. For this reason, biologists publishing stain formulae are urged to cooperate with the committee in putting the formulae on the basis of so many cubic centimeters of saturated solution. The saturated solution may be either aqueous or alcoholic, according to the needs of the individual case. Objections are sometimes made to this type of formulae in that it is difficult to prepare without waste when the solubility of a stain is not known. This is not a valid objection, however, because a saturated solution of any stain can always be kept on hand with an excess of undissolved material at the bottom of the bottle, adding more water or alcohol from time to time as more stock solution is needed in preparing staining solutions. This type of formula, therefore, has so many advantages without serious disadvantages that its use is recommended in all possible cases.—*H. J. Conn, Chairman, Commission on the Standardization of Biological Stains.*

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# *The Journal of Laboratory and Clinical Medicine*

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No. 5

## *ORIGINAL ARTICLES*

### NEW EXPERIMENTS WITH VAUGHAN'S CRUDE SOLUBLE POISON\*

BY FRANK P. UNDERHILL, PH.D., AND ROBERT KAPSINOW, M.D.

THE toxicity of Vaughan's Crude Soluble Poison is of great interest from the viewpoint of protein chemistry and its applications to the problems of serology. Irrespective of interpretations advances in our knowledge relative to either the chemistry of this remarkable preparation or its actions and transformations within the body are certain to lead to results pregnant with possible applications.

The present paper is the outcome of a series of observations, the continuation of studies carried through in this laboratory for a number of years. The particular problems which form the basis of this communication are in the first place the determination whether the results already published with respect to the toxicity of Vaughan's Crude Soluble Poison with the guinea pig as subject may be applied to the rat, and further modification of the preparation of the poison together with closer inspection of the fractions which accompany the poison.

#### PREPARATIONS

The preparations which are pertinent at present are as follows:

*Preparation A.*—Made from casein exactly according to Vaughan's original method except that the alkaline alcohol extract was allowed to cool before filtration. It will be recalled that in the original method the period of heating was one hour twice repeated with fresh portions of alkaline alcohol.

*Preparation B.*—Made from casein exactly as Preparation A except that the period of heating for each extraction was two hours instead of one hour.

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\*From the Department of Pharmacology and Toxicology, Yale University, New Haven.  
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*Preparation D.*—The method given for Preparation A was exactly followed with coagulated egg white.

*Preparation F.*—Vaughan's nontoxic residue from the preparation of the poison from casein was treated as in the preparation of Preparation A.

*Preparation G.*—Made exactly as Preparation A except that the alkaline alcohol mixture was filtered hot instead of filtering after cooling.

These preparations served as the starting point for the determination of the toxicity experiments. Other preparations employed will be explained in connection with the individual substances above.

## OBSERVATIONS\*

*Preparation A.*—From the data presented in Tables I and II it may be observed that Vaughan's Crude Soluble Poison possesses for the rat a toxicity

TABLE I

PREPARATION A. VAUGHAN'S CRUDE SOLUBLE POISON  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 3, 1922.			
RAT*	WT.	DOSE PER 100 GMS.	INJECTED
	gms.	mgms.	P. M.
1	245	10	2:39
2	330	10	2:40
3	270	15	2:40
4	300	15	2:40
5	260	20	2:41
6	150	20	2:41
7	250	25	2:42
8	250	25	2:43
9	215	30	2:44
10	295	35	2:45

At 3:00 P. M. all animals seemed quiet and indisposed to move or be moved.

\*All recovered in 2 hours.

TABLE II

PREPARATION A. VAUGHAN'S CRUDE SOLUBLE POISON  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 6, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	165	25	3:07		1
2	240	25	3:08		1
3	245	50	3:10	29 min.	
4	270	50	3:09	53 "	
5	265	100	3:11	47 "	
6	125	100	3:13	26 "	
7	165	125	3:14	23 "	
8	120	125	3:15	20 "	
9	115	150	3:16	16 "	
10	132	150	3:17	15 "	

Death—respiratory with convulsions. Nose, feet and ears became cyanotic.

Autopsy—few intestinal hemorrhages. Lungs collapsed, seemed normal.

\*The rats employed were obtained from the experimental colony of Osborne and Mendel. Generally the substances injected were dissolved in physiological salt solution or water without changing the reaction of the substance. When insoluble in these solutions a faintly alkaline solution was employed.

which is quite comparable with that for the guinea pig. The rat may therefore be employed in experimentation with this substance. In doses up to 25 mg. per 100 gm. of rat this particular preparation of Vaughan's Crude Soluble Poison was nonlethal. It was rapidly lethal, however, in doses of 50 mg. per 100 gm. of rat and above, death occurring more rapidly with increase of dosage.

*Preparation A(B).*—This substance comprised the HCl + NaCl precipitate forming on neutralization of the alkaline alcohol solution. According to the toxicity figures in Table III it possesses relatively few poisonous properties even in doses as high as 200 mg. per 100 gm. of rat.

*Preparation A(C).*—This preparation was that portion of the residue from the alcoholic distillation insoluble in absolute alcohol. Little or no toxicity

TABLE III

PREPARATION A(B). THE HCl + NaCl PRECIPITATE FORMED ON NEUTRALIZATION OF THE ALKALINE ALCOHOL SOLUTION

TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 30, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	140	50	3:44		1
2	105	100	3:44		1
3	140	150	3:45	Found dead 9:30 P. M.	
4	105	200	3:45		1

Animals soon became very prostrated, nose became cyanotic and they were weak on the feet.

TABLE IV

PREPARATION A(C). PORTION OF RESIDUE FROM ALCOHOL DISTILLATION INSOLUBLE IN ABSOLUTE ALCOHOL

TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 30, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	RECOVERED	
	gm.	mg.	P. M.		
1	135	50	3:02		1
2	100	100	3:02		1
3	90	150	3:03		1
4	115	200	3:05		1

Not affected—no symptoms.

is possessed by this substance—see Table IV—even in relatively large doses—200 mg. per 100 gm. of rat.

*Preparation A(D).*—Portion of A(B) insoluble in absolute alcohol. From Table V it is apparent that large doses of this preparation are without noticeable effect.

*Preparation A(E).*—Portion of A(B) soluble in absolute alcohol. Doses up to 200 mg. per 100 gm. of rat are nontoxic. See Table VI.

From these observations it is evident that of fractions formed during the preparation of this sample of Vaughan's Crude Soluble Poison none exhibited

evidence of toxic properties except the presumably toxic fraction, namely, Vaughan's Crude Soluble Poison.

*Preparation B.*—This preparation was made in exact accordance with Vaughan's directions except that the heating was carried through for a period of two hours instead of one hour. The data in Table VII demonstrate that this preparation of Vaughan's Crude Soluble Poison is not as toxic as Preparation A. It is quite possible that the extra period of heating is responsible for this difference although different preparations of this substance formed from equal periods of heating show just as great variations in toxicity as may be seen from Vaughan's investigations and from former communications from this laboratory.

*Preparation B(A).*—Presumably comparable to Vaughan's nontoxic residue. Very unexpectedly this fraction gave evidence of distinct toxicity being fatal in doses of 100 mg. per 100 gm. of rat (Table VIII). It is quite possible that the longer periods of heating so altered the original substance as to change the solubility of unknown substances to a degree such that they were included in the nontoxic portion. This inclusion of toxic products in the presumably nontoxic residue may account perhaps for the lower toxicity of this preparation (Preparation B) of Vaughan's Crude Soluble Poison.

TABLE V

PREPARATION A(D). PORTION A(B) INSOLUBLE IN ABSOLUTE ALCOHOL  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 30, 1922.				
RAT	WT.	DOSE PER 100 GM.	INJECTED	RECOVERED
	gm.	mg.	P. M.	
1	130	50	2:48	1
2	75	100	2:49	1
3	110	150	2:50	1
4	100	200	2:50	1
No apparent symptoms. Animals appeared very thirsty.				

TABLE VI

PREPARATION A(E). PORTION A(B) SOLUBLE IN ABSOLUTE ALCOHOL  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 30, 1922.				
RAT	WT.	DOSE PER 100 GM.	INJECTED	RECOVERED
	gm.	mg.	P. M.	
1	110	50	2:53	1
2	80	100	2:53	1
3	115	200	2:55	1
4	95	200	2:45	1
No apparent symptoms.				

*Preparation B(W).*—The residue from dialyzed HCl + NaCl precipitate. This preparation shows a comparatively low toxicity since no apparent symptoms were to be observed with doses of 150 mg. per 100 gm. of rat. With a dose of 200 mg. one rat died during the night of injection. (See Table IX).

*Preparation B(W').*—Portion of B(W) soluble in absolute alcohol. This preparation was devoid of toxic effects even in relatively large doses since

200 mg. per 100 gm. of rat could be injected without apparent symptoms. (See Table X).

In summary, the striking feature of this preparation and its by-products is the comparatively high toxicity of that portion which presumably corresponds to Vaughan's nontoxic residue. The only explanation apparent is that the longer period of heating in its preparation possibly changed either the chemical character of this fraction or its solubility so that it is included in this fraction instead of with the toxic end product. This change in the toxicity of the

TABLE VII  
PREPARATION B. VAUGHAN'S CRUDE SOLUBLE POISON  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 4, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	
	gm.	mg.	A. M.		
1	225	10	10:46		
2	290	20	10:46		
3	225	30	10:47		
4	215	40	10:47		
5	150	50	10:48		
6	185	60	—		
7	235	70	10:50	3/5/22 Found dead 12:00 noon	
8(B)	290	80	—	3/6/22 Found dead 3:00 P. M.	
9	290	90	10:53	11:49 A. M.	
10	250	100	11:04	11:40 A. M.	
11(C)	260	110	11:05	3/6/22 Found dead 3:00 P. M.	
12(A)	205	120	11:05	11:14 A. M.	
13	180	130	11:06	3/8/22 Found dead	
14(B)	295	60	11:07	3/6/22 Found dead 2:00 P. M.	
15	240	80	11:08	11:46 A. M.	

Animals became sick at once with generalized weakness. They tend to fall to one side as if they had muscular weakness of the legs with occasional spurts of activity. Convulsions are rare.

(A) Autopsy showed abscesses in lungs, otherwise normal.

(B) Diffuse peritonitis, lungs negative.

(C) Diffuse peritonitis with intestinal perforation from Peyer's patch.

TABLE VIII  
PREPARATION B(A). VAUGHAN'S NON-TOXIC RESIDUE  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 2, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	130	50	4:04		
2	75	100	4:05	Found dead 5:30 P. M.	
3	110	150	4:07	Found dead 5:30 P. M.	
4	100	200	4:06	Found dead 8:00 P. M.	

Ears and nose became pale at once. At 5:30 Nos. 2 and 4 were very sick. Breathing was very rapid.

Autopsy showed intestines filled with fluid blood. Vessels of testicles deeply engorged. Liver apparently normal, very few adhesions. Heart normal. Lungs collapsed and pale.



nontoxic residue may account perhaps for the diminished poisonous effect exhibited by this preparation of Vaughan's Crude Soluble Poison.

*Preparation D.*—Vaughan's Crude Soluble Poison from coagulated egg white. It was of interest to determine the influence of previous heating of the protein upon the toxicity of the end product formed and the data for the toxicity of this substance may be found in Table XI. Even with doses of 25 mg. per 100 gm. of rat all animals were made very sick. There is, however, a sharp division between the fatal and the nonfatal dose and this varies some-

TABLE IX

PREPARATION B(W). RESIDUE FROM THE DIALYZED HCl + NaCl PRECIPITATE  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 2, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	135	50	3:53		1
2	100	100	3:54		1
3	90	150	3:55		1
4	115	200	3:56	Found dead 4/5/22 A. M.	

No apparent symptoms. Autopsy showed profuse peritoneal adhesions.

TABLE X

PREPARATION B(W"). THE PORTION OF B(W) SOLUBLE IN ABSOLUTE ALCOHOL  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 2, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED		RECOVERED
	gm.	mg.	P. M.		
1	110	50	3:59		1
2	80	100	3:59		1
3	115	150	4:00		1
4	95	200	4:00		1

No apparent symptoms.

TABLE XI

VAUGHAN'S CRUDE SOLUBLE POISON FROM COAGULATED EGG WHITE  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 8, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	P. M.	
1	265	25	2:52		1
2	265	25	2:53		1
3	265	50	2:54		1
4	230	50	2:55		1
5	140	100	2:55	3:10	
6	305	150	2:56	3:01	
7	345	125	2:57	3:01	
8	270	125	2:57	3:53	
9	305	150	2:59	3:04	
10	355	150	3:50		1

Animals very rapidly went into convulsions with emprostotonos. There was no apparent respiratory difficulty. Nose, ears and legs did not become cyanotic and the animals died after extreme convulsive attacks. Heart beat long after respiration ceased. At 4:00 P. M. all others were still very sick and did not move. Autopsy showed nothing.

where between 50 and 100 mg. the latter being invariably and very rapidly fatal. Apparently the physical and chemical change involved in the coagulation of egg white does not prevent, although it may modify, the degree of toxicity of the toxic end product.

*Preparation D(A).*—Presumably nontoxic residue. This substance is nearly as toxic as the end product (Table XII) judged from the lethal viewpoint, except that death is more delayed.

*Preparation D(B).*—The precipitate which collected in the filtrate after alcoholic solution had cooled. From the data (Table XIII) it is quite apparent

TABLE XII  
PREPARATION D(A). PRESUMABLY NONTOXIC RESIDUE  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 4, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	A. M.	
1	280	50	10:08		1
2	170	100	10:09	11:37	
3	225	150	10:10	11:40	
4	265	150	10:10	11:40	

Following injections animals screamed and seemed in pain. At 10:45 all animals were quiet, stupefied and inactive.

Autopsy showed intestines filled with blood. Abdominal walls were hemorrhagic.

TABLE XIII  
PREPARATION D(B). PRECIPITATE WHICH COLLECTED IN FILTRATE AFTER ALCOHOL  
SOLUTION COOLED  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 4, 1922.				
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED
	gm.	mg.	A. M.	
1	300	50	10:01	Found dead 8:00 P. M.
2*	285	100	10:02	Found dead 2:45 P. M.
3	200	150	10:04	2:45 P. M.
4	230	130	10:05	11:42 A. M.

Main symptom was weakness with cyanosis.

Autopsy showed hemorrhage in the intestines up to large gut. Liver, lungs and heart normal.

\*Found pregnant. Fetuses dead. Placenta very easily detached. Hemorrhage into the intestines and cecum very great.

that this substance possesses greater toxicity than the more soluble portion of Vaughan's Crude Soluble Poison from which it is separated. Although this preparation killed in smaller doses than Preparation D, death was delayed for a much longer period.

*Preparation—Residue 2''.*—This preparation was made by evaporating, on the water-bath, to dryness in acid solution the HCl precipitate obtained on neutralization of the alkaline alcohol solution. The dried residue was extracted with absolute alcohol and the alcoholic filtrate evaporated to dryness. Toxicity experiments (Table XIV) show a comparatively high grade toxicity being

more poisonous from the standpoint of deaths than the presumably Vaughan's Crude Soluble Poison of this preparation except that death was much less rapid.

*Preparation—HCl Precipitate Dialyzed—Residue.*—This preparation was toxic in comparatively large doses, see Table XV.

*Preparation D(X).*—Formed by extraction with absolute alcohol of the water insoluble residue of the Vaughan nontoxic portion D(A).

Even in doses of 50 mg. this substance is fatal (see Table XVI) although death takes place only after a number of hours.

From the experience gained with this series of preparations the query

TABLE XIV

PREPARATION RESIDUE 2". ABSOLUTE ALCOHOL EXTRACT OF HCl PRECIPITATE  
(ACID SOLUTION)

TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 4, 1922.				
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED
	gm.	mg.	A. M.	
1	200	50	10:15	2:45 P. M.
2	120	100	10:15	11:15 A. M.
3	225	150	10:16	11:15
4	305	135	10:16	11:05

Animals were all in pain but this seemed to last only for a short time then animals became very weak and cyanotic. At 10:45 all animals were very inactive. Died with a slight convulsion.

Autopsy revealed intestines filled with fluid blood. Abdominal walls were hemorrhagic. Liver appeared necrotic. Fat had a peculiar glistening appearance. Lungs contained few hemorrhagic points.

TABLE XV

HCl PRECIPITATE DIALYZED—RESIDUE

TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 4, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	A. M.		
1	160	50	10:29		1
2	170	100	10:29		1
3	210	150	10:30	Found dead 8:00 P. M.	
4	145	200	10:30	Found dead 2:45 P. M.	

Autopsy showed nothing abnormal.

TABLE XVI

PREPARATION D(X). ALCOHOL EXTRACT OF WATER INSOLUBLE PORTION OF NONTXIC RESIDUE  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

April 4, 1922.				
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED
	gm.	mg.	A. M.	
1	80	50	10:45	Found dead 8:00 P. M.
2	215	100	10:45	Found dead 2:45 P. M.
4	170	150	10:45	Found dead 2:45 P. M.

Autopsy showed nothing abnormal.

arises whether the toxicity exhibited by all of these products is due to the same substance or to different groups. It is of course quite possible that in this series, for unknown reasons, fractionation into toxic and nontoxic portions was not so sharp. Indeed this would appear to be the simplest explanation although only by further study can this point be determined.

TABLE XVII  
PREPARATION F. ORIGINAL NONTOXIC RESIDUE OF CASEIN  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 11, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	205	50	6:35		1
2	170	100	6:38		1
3	205	200	6:36	Found dead 9:00 P. M.	
4	205	250	6:41	Found dead 3/12/22 12:30	
5	160	300	6:42		1
6	190	350	6:45		1

Animals at first quite sick but at 9:00 P. M. seemed well except for a little weakness. Autopsy of No. 3 showed peritoneal hemorrhage and whole intestines filled with blood from ruptured vessel.

*Preparation F.*—Original nontoxic residue formed from casein by treatment according to Vaughan. This preparation possessed a low grade toxicity only, since in doses of 200 mg. and above, death occurred only after several hours (Table XVII).

*Preparation F(1B).*—With a view of testing the possibility of the formation of a toxic product by further treatment of the above (Preparation F) nontoxic residue, this substance was subjected to the action of the alkaline alcohol again, first for a period of six hours; the remaining residue for three hours and the final residue for a like period. Practically all of the material was finally rendered soluble in the alkaline alcohol solution. The united extracts were then carried through in the manner usual in the preparation of Vaughan's Crude Soluble Poison. The final preparation was called Preparation F(1B).

Comparing the toxicity of this substance with that of Preparation F it will be observed (Table XVIII) that the further treatment of the nontoxic residue has very greatly increased its toxicity, doses of 50 mg. per 100 gm. of rat proving rapidly fatal, death occurring sooner with increasing doses. It is therefore quite possible to form from the nontoxic residue a substance possessing poisonous properties to a degree equal to the crude soluble poison obtained from the untreated native protein. Such an observation lends support to the view that in the preparation of Vaughan's Crude Soluble Poison the protein molecule is not split into an inherent poisonous and a non-poisonous group but rather that toxic products may be formed by the treatment so long as any protein remains. In other words the treatment results in the formation of toxic substances presumably by hydrolytic change, perhaps similar in man-



ner to the formation of proteoses, from which indeed the crude soluble poison may be formed.

*Preparation F(1A).*—From the above treatment of the nontoxic residue there resulted the formation of a small quantity of material which would correspond to the nontoxic residue of Vaughan. Toxicity experiments (Table

TABLE XVIII

PREPARATION F(1B). FORMED BY FURTHER TREATMENT FROM THE NONTOXIC RESIDUE  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 8, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	A. M.	A. M.	
1	280	25	11:25		1
2	290	50	11:26	11:51	
3	290	75	11:27	11:35	
4	290	100	11:28	11:35	
5	290	125	11:29	11:34	
6	265	150	11:30	11:34	

Animals died in a similar manner to those injected with Preparation D. Toxic end product, i.e., convulsions with spastic movements and no real cyanosis or gasps even though the heart beat after respiration had ceased.

TABLE XIX

PREPARATION F(1A). CORRESPONDING TO RESIDUE OF VAUGHAN  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 17, 1922.				
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED
	gm.	mg.	P. M.	P. M.
1	135	50	2:46	Found dead 3/18/22 11:00 A. M.
2	175	100	2:47	4:50
3	180	150	2:48	4:45
4	200	200	2:50	5:02
5	170	250	2:52	4:06
7	140	350	2:55	3:55

Immediately after the injection the animals became furious and curled up about the point of injection as though in great pain. But after a short interval they seemed better. The last animal injected seemed to have rigid spastic paralysis of both hind legs from which it soon recovered. Animals died after a few slight convulsions and asphyxia.

Autopsy showed congested and injected intestines and in some places loops that were black and contained blood. This must have been produced by the injection, for lower down there was normal, formed feces. Lungs and heart were negative.

TABLE XX

VAUGHAN'S CRUDE SOLUBLE POISON FROM CASEIN  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 17, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	P. M.	
1	225	25	3:25		1
2	220	50	3:25		1
3	205	75	3:26		1
4	185	100	3:26	Found dead 3/18/22 11:00 A.M.	
5	240	125	3:27		1
No symptoms present.					

XIX) showed a relatively high grade toxicity, although death was distinctly delayed.

*Preparation G.*—Vaughan's Crude Soluble Poison from casein prepared by the treatment outlined for Preparation A except that in Preparation G the alkaline alcohol solution was filtered while hot. This substance was not very poisonous since one rat died with a dosage of 100 mg. per 100 gm. of rat on the day following the injection, whereas an animal receiving 125 mg. recovered (See Table XX).

*Preparation G (No. 1).*—Material which separated on cooling from the alcohol solution possessed a high degree of toxicity (see Table XXI) as did Preparation G.

TABLE XXI

PREPARATION G(No. 1). SUBSTANCE INSOLUBLE IN ALCOHOL ON COOLING  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 17, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	P. M.	
1	180	50	4:08		1
2	200	100	4:09	Found dead 3/18/22 11:00 A.M.	
3	165	150	4:10	Found dead 3/18/22 11:00 A.M.	
4	135	200	4:10	4:42 P.M.	
5	150	250	4:10	5:40 P.M.	
Animals reacted like those of Preparation F(1A) with reaction to point of injection. Autopsy findings similar to Preparation F(1A).					

TABLE XXII

NONTOXIC RESIDUE OF VAUGHAN  
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 27, 1922.					
RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	P. M.	
1	245	25	3:45		1
2	250	50	3:47	Found dead 3/28/ in A.M.	
3	305	75	3:47	6:40	
4	200	100	3:49	6:40	
5	255	150	3:50	6:40	
6	135	200	3:50	5:00	
Symptoms and autopsy findings as in G(No. 1).					

*Preparation G (No. 2).*—Presumably nontoxic residue of Vaughan. Toxicity experiments show this preparation to possess distinctly poisonous properties since doses of 50 mg. or above proved fatal although death occurred with distinct slowness. On the other hand it possessed toxicity of a grade equal to or greater than the corresponding crude soluble poison obtained in the same preparation (see Table XXII).

From these latter preparations it must be quite apparent that a distinct separation of a toxic from a nontoxic portion by treatment with alkaline alcohol is not always effected.

## SUMMARY

The preparation known as Vaughan's Crude Soluble Poison possesses for the rat a degree of toxicity similar to that for the guinea pig. The former animal may therefore be employed as experimental subject and so far as toxicity is concerned the interpretations derived from the one are directly applicable to the other animal.

The toxicity of various preparations of Vaughan's Crude Soluble Poison vary in degree whether made under similar or different experimental conditions. The division of the substance resulting from the treatment into toxic and nontoxic fractions is not uniformly sharp, since the so-called nontoxic residue may possess distinctly poisonous properties. Modifying the period of heating radically alters the toxicity of the products obtained, the general tendency being for a decrease in the toxicity of that portion known as Crude Soluble Poison and an increase in the toxicity of the so-called Nontoxic Residue. The whole matter may perhaps be explained on the basis of a change in solubility whereby under altered conditions certain substances are included in one or another fraction.

Subjecting coagulated egg white to the treatment employed in the formation of Vaughan's Crude Soluble Poison results in obtaining a preparation of high grade toxicity. It is noteworthy that all the various by-products fractionated in the formation of this substance gave evidence of toxic properties. This applies to the so-called nontoxic residue which was nearly as poisonous as the toxic end product. Apparently the change induced by the process of coagulation of the egg white facilitated rather than diminished the formation of toxic substances.

Vaughan's Crude Soluble Poison may be formed from the so-called nontoxic residue by further treatment.

The observations recorded above lead to the view that the substance or substances included in the term "Vaughan's Crude Soluble Poison" are products formed presumably by progressive hydrolytic change rather than that the protein molecule is split into a toxic portion and a nontoxic residue. On such an hypothesis may be readily explained the varying toxicity of different samples of Vaughan's Crude Soluble Poison, and the observation that by altering environmental conditions changes in toxicity of various fractions may be obtained.

It is therefore quite probable that Vaughan's Crude Soluble Poison cannot be regarded as a chemical entity but must rather be looked upon as a mixture of substances varying according to the conditions leading to its formation.

# ORGANIC, PROTEIN AND COLLOIDAL SILVER COMPOUNDS; THEIR ANTISEPTIC EFFICIENCY AND SILVER-ION CONTENT AS A BASIS FOR THEIR CLASSIFICATION\*

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## INTRODUCTION

THE practical value of the high antiseptic efficiency of silver nitrate is limited by its side-actions: irritation and pain, astringency and corrosion. These may be largely avoided by the use of colloidal silver compounds which combine in many instances a fair degree of antiseptic action with a much smaller degree or entire absence of the irritant side-actions. The irritant and antiseptic actions of silver nitrate are due essentially to the free silver ions. The antiseptic action of the colloidal preparations has also been attributed to the presence and liberation of low concentrations of silver-ions, the concentration being so low as to be practically nonirritant, but still sufficient to be more or less antiseptic. Dreser<sup>1</sup> for instance, showed that the antiseptic action of the commercial "colloidal metallic silver" is destroyed if it is treated by agents that reduce silver ions to metallic silver (zinc dust, hydroquinon, pyrogallol); or by agents that bind silver ions (sodium thiosulphate, potassium cyanide, or sodium chloride). Gros<sup>2</sup> concludes that the colloidal silver preparations, notwithstanding their low concentration of silver ions, may be more efficiently antiseptic, in the presence of sodium chloride, than is silver nitrate, because the silver chloride from the colloidal silver forms a finer precipitate and therefore redissolves more readily than when it is precipitated from silver nitrate (except in very dilute solutions). This, however, could come into play only under certain conditions, notably with prolonged contact.

The numerous commercial brands of colloidal silver compounds may be grouped under a limited number of types.<sup>3</sup> Clinical experience has shown that these types differ in irritation and in antiseptic efficiency, so that their therapeutic field is not quite identical; but no exact comparisons have been made, for want of suitable quantitative methods. Even the classification of individual products, which should reflect the therapeutic grouping, was sometimes doubtful. Experiments on the restraint of bacterial growth have been made under various conditions by many observers; but the results have been contradictory, probably because of technical difficulties, so that they have but little value as a guide either to therapeutics or to classification. An encouraging prospect was opened by the observation of Dreser<sup>1</sup>

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that the growth of ordinary yeast furnishes an accurate and convenient index of silver ions, of antiseptic efficiency, and of irritation. Dreser applied the method to "colloidal metallic silver" preparations, and suggested that it might be useful for quantitative comparisons of organic silver compounds with silver nitrate; but he did not elaborate it further. Our experience with this method, somewhat modified as described in the appendix, shows that it furnishes reliable criteria for the comparative antiseptic efficiency of silver compounds, inorganic, protein and colloidal. The technic is simple and the data are quantitative, objective and of adequate uniformity. By determining the antiseptic efficiency in watery mixtures and in normal saline solution data are obtained which have a direct bearing on the various types of clinical application of silver salts; and which, moreover, indicate the relative proportion of ionized (irritant and astringent) silver; nonionized (nonirritant) but antiseptic silver, and inactive or reserve silver. We shall discuss this more fully later.

#### PRODUCTS EXAMINED

Silver nitrate was taken as the fixed standard and control. With this were compared fourteen organic or colloidal compounds, including the prominent commercial brands. Two or more specimens of each preparation (with one exception) were used, to determine the variability of the brands, making a total of 34 samples. Some were purchased recently, in unopened bottles; others were older specimens, on hand either in this or in the American Medical Association laboratory. These furnished the opportunity to compare the older and new preparations.

#### ANTISEPTIC EFFICIENCY IN AQUEOUS SOLUTIONS

This represents the *total* activity of ionized and nonionized silver. The measure of the activity is the "*inhibiting dose*," namely, the quantity of the compound in milligrams per 10 c.c. of mixture that is just sufficient to prevent gas formation within an hour, under the conditions of the experiment. This may also be stated as the "*inhibiting concentration*," 1 mg. per 10 c.c. representing a concentration of 1:10,000, etc. Silver nitrate was used for a comparison and as a check of correct technic since it gives reasonably uniform results: 0.25 mg. (1:40,000) nearly always prevented gas formation and was accepted as the standard; 0.22 to 0.20 may or may not be completely effective; 0.17 mg. always permits a slight formation of gas. Exceptionally a specially active yeast is found, which requires a higher concentration than 0.25 mg. In that case, a corresponding correction is made for the product with which it is to be compared.

The percentage variation of different tests of the other compounds was generally also small. It was not attempted to reduce it to less than 25 per cent from their mean, since this range is very much smaller than the difference between the various groups of compounds; the inhibiting dose ranging from 0.25 mg. (1:40,000) for silver nitrate, to 150 mg. (1:67) for one specimen of silvol. The data for the individual products and specimens are shown in columns 2 and 4 of Table I.

Different specimens of a given brand show satisfactory agreement, usually within 25 per cent, at most 50 per cent, of the smaller figure, for all the commercial products except those of the "Protargin Mild" (Argyrol) type, i.e., Group V of the Table. For these the strongest samples are two or three times as active as the weakest. In five of the six brands the recently purchased sample was the weaker. We cannot say whether this is more than a coincidence.

The Table shows that silver compounds arrange themselves, according to their total activity, into five distinct groups, separated by relatively wide

TABLE I  
INHIBITING DOSES AND INHIBITING CONCENTRATIONS OF THE SPECIMENS

1. PRODUCT	2. WATER	3. NORMAL SALINE (0.8% NaCl)	4. WATER	5. NORMAL SALINE (0.8% NaCl)
	mg. per 10 c.c.	mg. per 10 c.c.	1:	1:
<i>Group I.</i> Silver nitrate	0.25	20	40000	500
<i>Group II.</i> "Silver nucleinate"				
Roche.	1.0	300	10000	30
Sophol. x	0.85	125	11500	80
Sophol. y	1.0	100	10000	100
Albargin 1922 (Silver gelatose)	1.2	—	8333	—
Mean of Group II	1.0	125	10000	70
<i>Group III.</i>				
Protargol (1)	2.2	110	4500	90
Protargol w	2.0	60	5000	165
Protargol 4x	2	125	5000	80
Protargol 5x	2.5	120	4000	85
Proganol /	2.5	100	4000	100
Proganol y	3.0	65	3300	155
Protargentum x	2.0	35	5000	280
Protargentum l	2.0	70	5000	140
Protargentum A	3.0	50	3300	200
Silver proteinate	2.0	150	5000	65
Mean of Group III	2.0	95	4500	105
<i>Group IV.</i>				
Collargol x	10.	85	1000	115
Collargol /	11.	100	910	100
Mean of Group IV	10.5	93	955	108
<i>Group V.</i>				
Vargol X	11.	35	910	280
Vargol y	20.	45	500	220
Solargentum (1)	15.	40	670	250
Solargentum 2x	20.	60	500	165
Solargentum x	55.	80	180	125
Argyn (1)	20.	100	500	100
Argyn x	30.	75	330	130
Argyn y	55.	60	180	165
Cargentos (new)	35.	100	280	100
Cargentos (new process)	60.	—	167	—
Argyrol (1)	40.	500	250	20
Argyrol y	70.	40	145	250
Silvol x	60.	150	165	65
Silvol (1)	75.	100	135	100
Silvol (2x)	125.	175	80	55
Silvol of Current Sample	150.	—	67	—
Mean of Group V	38.	78	305	128

gaps. These are recapitulated in Table II. They correspond very satisfactorily to the generally accepted clinical types and clear up the position of some of the doubtful products. The "total activity" test is therefore an adequate and very convenient objective criterion for the grouping of the silver products.

The data have a further practical value in that they reflect the relative therapeutic antiseptic efficiency or the relative concentration of the solutions that must be used to secure equal antiseptis within an hour, provided

TABLE II

THE GROUPING OF SILVER COMPOUNDS ACCORDING TO THEIR ACTIVITY IN AQUEOUS SOLUTIONS

GROUP	COMPOUNDS	INHIBITING QUANTITY IN MG.	INHIBITING CONCENTRATION
I. Inorganic salts.	Silver nitrate	0.25	1:40,000
II. Protargin fortissimum.	Silver nucleinate Sophol Albargin	0.85-1.2	1:13,300 to 1:8,333
III. Protargol.	Protargol Proganol Protargentum Silver proteinate	2-3 (mean 2.2)	1:5,000 to 1:3,300
IV. Collargol.	Collargol	10-11	1:1,000 to 1:900
V.* Argyrol.	Vargol Solargentum Argyn Cargentos Argyrol Silvol	11-150 (mean 38)	1:900 to 1:67

\*Group 5 may be thought to contain members with too wide a range; it is, however, but little greater than the difference in different lots of the same product.

that the surfaces are fairly clean, or when the solutions are used by lavage. The prophylactic efficiency against gonorrheal infection comes under this heading, for there the action must be exerted within a few minutes after application to secure the maximum protection.

To facilitate the use of the data for therapeutic purposes, Table III has been prepared, showing the concentration of the various groups that is equivalent in antiseptis to a 1:1000 solution of silver nitrate. The activity in a saline medium is also shown for convenience and will be discussed in the next section.

TABLE III

CONCENTRATIONS OF SILVER COMPOUNDS EQUAL IN ANTISEPTIC VALUE TO SILVER NITRATE 1:1000

GROUP	DISSOLVED IN WATER	IN PRESENCE OF 0.8 PER CENT NaCl.
I. Silver nitrate	1:1000	1:1000
II. Protargin fortissimum	1:250	1:140
III. Protargol type	1:115	1:210
IV. Collargol	1:25	1:216
V. Argyrol type	1:7.6	1:256

## ANTISEPTIC EFFICIENCY IN SALINE SOLUTION

The presence of sodium chloride decreases very greatly the antiseptic efficiency of silver nitrate; to a smaller extent that of the organic silver compounds. This, of course, is due to the precipitation of the silver as chloride, and the decrease is proportional to the silver-ion content of the aqueous solution. However, silver chloride is not absolutely insoluble; a small quantity escapes precipitation or is redissolved, and continues to act as an antiseptic. This solution is favored by the presence of colloids, and for this reason the colloidal silver compounds, with their protective proteins, may be more effective than silver nitrate, as pointed out by Gros. However, this occurs probably only under special conditions, namely when the quantity of active silver is so small that hours or days are required to check bacterial growth. This would be valuable in clinical conditions that permit practically continuous application; and it would then have the material advantage that the nonionized silver is nonirritant. To this we shall recur later.

The inhibiting dose and concentration of our silver compounds in the presence of sodium chloride is shown in columns 3 and 5 of Table I. A mere glance at column 5 shows that the activity of all the groups, except silver nitrate, has been reduced to a nearly uniform level. The inhibiting concentration ranges between 1:280 and 1:20, without any reference to the groups. The mean is just 1:100; and half of all the specimens lie between 1:80 and 1:125.

This means that the interference of the chloride is proportional to the activity of the preparation in watery solution, which, again, merely means that reduction of activity is due to the inactivation of silver ions. What remains is the activity of the silver chloride, and it is interesting and of practical importance to note that nearly the same amount of this is dissolved for equal weights of all the compounds, so that the nature of the compound itself is practically negligible. The only notable exception is silver nitrate, which, contrary to expectations, retained an activity of 5 times the average figure. Presumably the preponderance of silver ions is so great, with this salt, that some remained free, in accordance with the law of mass action.

## RATIO OF IONIC AND NONIONIC ACTIVE SILVER

Table III shows that, if applied in normal saline for an hour, silver nitrate 1:1000 is equivalent to the colloidal protein-silver preparations in the concentration of about 1:250. Clinically, however, silver nitrate is not applied in saline solution, but in water; and before it meets with enough chloride to dis-ionize it, it has caused so much irritation that it cannot be used frequently. The protein compounds, on the other hand, cause so little irritation that they can be applied continuously in much stronger solution. Clinically, therefore, the comparison is more likely to be, not with  $\frac{1}{4}$  per cent, but with 5 or 10 per cent protargin strong, or 10 to 40 per cent of protargin mild. For continuous application these would be superior.

The reason for this superiority, then, lies in a more favorable ratio, not of absolute efficiency, but of efficiency in proportion to irritation. This in turn rests on the ratio of nonionic but active silver, to ionic silver. The higher this



ratio, the more suitable is the compound for continuous application. The ratio is reflected by dividing the antiseptic efficiency in normal saline (representing the nonionic active silver) by the efficiency in water (representing active non-ionic plus ionic silver); or conveniently, by using Table I, and dividing column 2 by column 3. The quotients for the mean data of the groups are shown in Table IV.

TABLE IV

QUOTIENT OF NONIONIC TO TOTAL SILVER, REPRESENTING ALSO THE RATIO OF ANTISEPTIC EFFICIENCY TO IRRITATION

Group I.	Silver nitrate	0.0125
Group II.	Protargin fortissimum	0.008
Group III.	Protargol type	0.0023
Group IV.	Collargol	0.1
Group V.	Argyrol type	0.5

The silver nitrate probably shows a more favorable figure than it really deserves, for the reasons explained above (imperfect precipitation of silver ions); but the clinical superiority of the argyrol type over the protargol and other types, where irritation has to be avoided, is faithfully reflected. The protargol type can be rendered as nonirritant as the argyrol type by being dissolved in saline solution; but this of course involves a proportional loss of antiseptic activity, to about  $\frac{1}{50}$ th of the activity in water. In other words, solution in normal saline converts the protargol type of silver into the argyrol type of silver. It does not, of course, change the protein, and therefore does not fully reproduce the demulcent actions of argyrol.

#### INACTIVE OR RESERVE SILVER

If one calculates the quantity of silver nitrate that would produce the antiseptic effect of one gram of the colloidal preparation and subtracts the weight of silver in this quantity of silver nitrate from the total weight of silver in one gram of the colloidal preparation, the remainder represents essentially silver that does not participate in the antiseptic action (in one hour under standard conditions), and which is therefore *inactive silver*. We are not prepared to say whether this is entirely useless, or whether it may be slowly converted into active silver, and thus play a part in very prolonged contact with the tissues, either directly (analogous to the experiments of Gros), or by replacing part of the active silver that becomes inactive by absorption or by conversion into a permanently insoluble form, etc. This would be more properly termed "reserve-silver." This phase of the subject needs further investigation.

#### RESIDUAL BALLAST OR VEHICLE

This includes the total nonsilver part of the compound, i.e., what remains when the silver-content is subtracted from the total weight. It includes the oxygen of the silver oxide, the protein of the silver proteinate, and the protein present as stabilizer or as diluent. The protein has considerable practical importance; as a demulcent in local application; as a dangerous provocative of anaphylactoid reactions in patients with specific protein-hypersusceptibility

or on intravenous injection. Such instances have been reported; but the subject lies too far outside of the scope of the present investigation to justify further discussion.

#### PERCENTAGE OF THE VARIOUS FORMS OF SILVER

This was calculated for each specimen by the methods explained in the appendix, but only the median figures for the five groups need be reproduced. They are shown in Table V.

TABLE V  
MEDIAN PERCENTAGE OF THE VARIOUS FORMS OF SILVER

		IONIC	NONIONIC	TOTAL			
		SILVER	ACTIVE	ACTIVE	INERT	TOTAL	BALLAST
			SILVER	SILVER	SILVER	SILVER	
Group I.	Silver nitrate type	62.7	0.8	63.5	0.	63.5	36.5
Group II.	Protargin fortissimum	16.7	0.07	16.8	3.2	20.	80.
Group III.	Protargol type	7.05	0.21	7.26	1.04	8.3	91.7
Group IV.	Collargol type	1.34	0.18	1.52	76.5	78.	22.
Group V.	Argyrol type	0.32	0.2	0.55	20.5	21.	79.

This Table illustrates the progressive decrease of ionic silver and consequently of irritation and activity from the silver nitrate to the argyrol group; the uniformly small activity of the nonionic silver; consequently, the total activity practically parallel to the ionic silver; the independence of the inert silver from the other forms; and the variability of the ballast-content.

#### CONCLUSIONS

1. The antiseptic value of silver salts and organic or colloidal silver compounds may be determined by their inhibiting action on the yeast-fermentation of sugar.

2. Determinations with watery solutions permit the grouping of the commercial products into five types, shown in Table II, which correspond with the clinical groups.

They, therefore, furnish a satisfactory laboratory method for assigning new compounds to their proper groups.

They are also a useful check on the uniformity of different specimens of the products. The only brands that showed much variation belonged to the protargin mild (argyrol) type.

The determinations in water would be fairly parallel to the immediate or early antiseptic effects, especially on clean surfaces or with lavage.

3. The presence of sodium chloride reduces the activity of all the compounds to a low level, which is practically the same for all the compounds. The difference between the activity in water and the activity in saline corresponds to the content of free silver ions and is a measure of the irritant and astrigent effects.

The activity in saline solution is probably more nearly representative of the clinical conditions of prolonged contact.

The tables show the data for individual specimens and groups. The methods and further details are explained in the appendix.

## APPENDIX

In this have been included such details as would not have much interest for the general medical reader, but which serve to establish the validity of the method, or which deserve preservation for other reasons.

## 1. TECHNIC OF THE DETERMINATIONS

The materials needed are a constant temperature water-bath, ordinary test tubes (1.5 x 15 cm.) and smaller test tubes (0.8 x 10 cm.); a 10 per cent solution of cane sugar; fresh cakes of compressed yeast (Fleischmann's); solutions of the drugs to be examined (10-20 per cent); pipettes; chloride-free water. Make a 4 per cent yeast sugar mixture by triturating a weighed quantity of yeast in the 10 per cent sugar solution, place 10 c.c. portions of the mixture into a series of the large test tubes in racks; add the desired quantity of the drug in solution (preferably 1 c.c. or less) to the mixture; shake well; fill a small test tube with the mixture from the large test tube and at once invert it into the larger, being careful to allow no air bubbles to rise into the small tube; this is readily accomplished by rapidly inverting the small tube and dropping it into the solution in the large tube, although occasionally the presence of air bubbles necessitates refilling the small tube; place the tubes in the rack into the water-bath at 38° C. In one hour measure the amount of gas formed in the small test tube in centimeters. The reading should be done within one or two minutes after taking the tubes from the bath, to obviate contraction of the gas by cooling.

A control test should always be made with silver nitrate, 15 mg. and 25 mg. per 10 c.c. If 15 mg. should give us gas; or should 25 mg. give more than a trace of gas, the inhibitory dose of silver nitrate for that yeast must then be determined, and the reading for the compound corrected by the formula: True inhibiting dose of compound: determined inhibiting dose of compound :: 25: determined inhibiting dose of silver nitrate. For example a certain sample of yeast was inhibited by 250 mg. of silvol and by 40 of silver nitrate. The true inhibiting concentration of silvol was therefore

$$\frac{X}{250} = \frac{25}{40} ; X = \frac{25 \times 250}{40}$$

The experiments in sodium chloride solution are made in a similar manner by substituting 0.8 per cent sodium chloride for water.

A 4 per cent yeast suspension in 10 per cent cane sugar solution was used, as these percentages gave a suitable amount of gas formation in one hour; with a lower yeast concentration (2 per cent) the fermentation was delayed and made the experiments extend over an unnecessarily long time (some two hours) to give a similar gas formation. Fresh yeast suspensions were used for each experiment as on standing at room temperature for an hour or less sufficient gas was formed in the mixture to vitiate results.

Preliminary experiments were made with each sample of drug using a wide range of dosage such as 1, 2, 4, 8, 16, 32 mg., etc.; and then the more accurate dosage was determined, with these results as a basis. The readings were made in centimeters of gas formed and not in the actual quantity by

volume, as the experiments were comparative, and therefore, the relative amount of gas formed under the action of the different drugs was desired and not the actual amount.

A. *Accuracy of the Method.*—Control experiments, i.e., with yeast and sugar only, were made in all cases and from a series of 61 of these it was seen that the natural experimental variations were practically negligible, with the average  $\text{CO}_2$  formation of about 7 cm., with the extremes of 4.3 to 9 cm. of  $\text{CO}_2$ ; 24 of these were within 7 to 8 cm., 13 within 6 to 7 cm. and the remainder somewhat below or above these quantities.

B. *Effect of NaCl on Control growth without drugs*; NaCl up to 3 per cent has no appreciable effect on  $\text{CO}_2$  formation. No experiments were made with higher concentrations.

C. *Presence of NaCl in Materials Used.*—As traces of sodium chloride might interfere by precipitating some of the silver, chloride-free water was always used; the yeast sugar mixture contained mere traces of chloride in the filtrates and it will be seen later that such traces have no appreciable effect on the silver action.

D. *Effect of Dilution on  $\text{CO}_2$  Formation.*—The addition of 1 c.c. of water (10 per cent) has no effect; 1.5 c.c. may cause a slight decrease in  $\text{CO}_2$  formation, still within normal variations, but above that there is a readily demonstrable decrease. However, when it was necessary to add the drug in 1 c.c. or greater quantities, the drug was first put into the test tube, diluted to 5 c.c. and 5 c.c. of a double strength sugar-yeast mixture added (20 per cent sugar solution, 8 per cent yeast); this gave the usual 10 per cent sugar and 4 per cent yeast mixture and avoided any possible effect of dilution.

## 2. DETERMINATION OF THE SODIUM CHLORIDE CONCENTRATION THAT NEUTRALIZES THE INHIBITORY QUANTITY OF THE SILVER PREPARATIONS

In the saline solution (0.8 per cent) which was used in this work the sodium chloride was in large excess over the silver content of any of the compounds examined, including  $\text{AgNO}_3$ , so that it seemed worth while to determine the concentration of sodium chloride that would just neutralize the silver in the inhibiting quantity. The question was approached in two ways; first mixing the chloride and silver preparations and allowing them to stand for 10 minutes to 2 hours before adding the yeast sugar mixture; secondly, by adding the silver preparation to the yeast sugar mixture made up in saline solution of the desired strength. The first method was tried as it was thought that part of the silver might act on the yeast before combining with the sodium chloride. In either case a very large excess of sodium chloride was necessary to have an appreciable effect on the action of silver nitrate and protargol, which were taken as examples of the silver compounds. This is shown in Table VI. When silver compounds and chloride solutions were previously mixed somewhat smaller quantities of sodium chloride neutralized the silver, but a large excess of the chloride was still necessary.

The results indicate that under the experimental conditions the compounds formed by silver and sodium chloride may be actively antiseptic: a question which we have stated is not the province of this paper to discuss.



TABLE VI  
DECREASE OF ANTISEPTIC ACTIVITY ACCORDING TO EXCESS OF SODIUM CHLORIDE

EFFECT OF SODIUM CHLORIDE ON THE SILVER COMPOUND ACTIVITY	IONIC SILVER ATOMS: CHLORIDE ATOMS AS 1:	
	SILVER NITRATE	PROTARGOL
Negative or slight decrease	25	15
Moderate decrease	40	30
Large decrease	100	60+
Abolition of activity	200	200-300

### 3. CALCULATION OF THE TYPES OF SILVER

(a) *Total Silver*.—The percentage stated by manufacturers was accepted, since this has been repeatedly verified by independent workers.

(b) *Active Silver*.—The inhibiting dose of silver nitrate contains 0.159 mg. of silver. This was taken as the unit of "active silver," i.e., the quantity of the compound that just inhibited yeast was considered to contain 0.16 mg. of active silver. For instance, the inhibiting dose of a sample of protargol was 2.0 mg.; the percentage of active silver in the sample would therefore be

$$\frac{100 \times 0.16}{2} = 8.0 \text{ per cent.}$$

(c) *Inactive or Reserve Silver*.—This is obtained by subtracting the active silver (b) from the total silver (a). In the above example, this is  $8.3 - 8.0 = 0.3$  per cent.

(d) *Ionic Silver*.—This corresponds to the quantity of "active" silver that is destroyed by sodium chloride. In the protargol sample quoted the inhibiting dose in water was 2 mg.; in normal saline it was 125 mg. Therefore, the chloride destroyed the activity of  $\frac{123}{125}$  of the active silver. Since the total active silver was 8.0 per cent, the ionic silver amounts to  $8 \times \frac{123}{125} = 7.8$  per cent.

(e) *Nonionic but Active Silver*.—This corresponds to the difference between the total active and the ionic silver; in our example, this is  $8.0 - 7.8 = 0.2$  per cent.

(f) *Ballast or Vehicle*.—This includes all components except silver, and is obtained by subtracting the percentage of total silver from 100; in the example,  $100 - 8.3 = 91.7$  per cent.

### 4. OLD SOLUTIONS

Solutions of these preparations may preserve their activity fairly well as is shown by the fact that a 25 per cent solution of solargentum that was in the laboratory for about four years lost but a third of its activity (the inhibiting dose increasing from 15 mg. of a fresh solution of the same preparation, to 20 mg. in case of the old solution). On the other hand, they may deteriorate considerably for a 5 per cent solution of protargentum lost much of its activity in the same time; the inhibiting dose increased from about 2 to 8 mg.

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NOTES ON D'HERELLE'S PHENOMENON. ADAPTATION OF  
BACTERIOPHAGE ANTAGONISTIC TO BACILLUS DYSEN-  
TERIAE AND OTHER BACILLI TO VARIOUS COCCI.  
DEVELOPMENT OF A POLYVALENT BACTERIOLYSANT\*

(Preliminary Report)

BY EARL B. MCKINLEY, A.B., M.D., ANN ARBOR, MICH.

ACCORDING to D'Herelle, perhaps the first demonstration of the phenomenon of "bacteriophagy" was made by Hankin, who in 1896 reported the bactericidal action of the water of the Ganges and Jumna rivers in India for the cholera vibrio. With others it has usually been assumed that Twort was the first to describe this phenomenon since Hankin was unable to destroy the bactericidal power of the water by heating to 115° C. for a half hour in an autoclave, a temperature which destroys bacteriophages. It is not impossible, however, that the water from the rivers Hankin examined contained bacteriophages as the phenomenon has been demonstrated by Dumas in filtrates of water from the Seine. In recent years the literature dealing with this very interesting phenomenon has increased steadily. Investigators in this field have dealt with many different phases of the subject, including sources of bacteriolyants; variation in titer; effects of chemicals, temperature and concentration on the degree of lysis; nonspecificity of the filterable "substance"; separation of cultures into resistant and sensitive types by action of bacteriophages; inoculations in animals, therapeutic action, etc.

In 1915 Twort noted in a culture of staphylococci, transparent areas in which no cocci grew. Touching one of these transparent areas with a sterile platinum loop and then drawing the loop across the surface of a 24-hour agar culture of staphylococci, he found after a few hours, a streak marking the track of the loop which had become clear and transparent. Filtering this material from these transparent areas through a Berkefeld, he found that the filtrate would dissolve and kill most of the organisms in fresh staphylococcus agar cultures even in dilutions of one to a million. This work was confirmed recently by Gratia and he called attention to the fact that this is the only gram-positive organism for which an observation of transmissible autolysis has been made.

Bruynoghe and J. Maisin have also worked with staphylococci and state in one of their reports that the staphylococcus bacteriophage could not be made to have any effect upon either the typhoid group of *B. dysenteriae*. These investigators have worked with bacteriolyants which have been obtained from cultures of staphylococci while the following experiments are

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based upon the adaptation of bacteriolysants antagonistic to *B. dysenteriae* and other bacilli to various cocci. D'Herelle stated that there is only one species of bacteriophage and this can acquire activity against any organism. He found that a bacteriophage in addition to its lytic power against the organism causing the patient's infection could acquire a lytic activity against *B. proteus*, *B. coli*, *B. typhi murium*, *B. typhosus*, *B. paratyphosus* A and B, *B. diphtheriae*, *B. gallinarum*, *B. subtilis*, *V. cholerae*, staphylococci and other organisms.

The material used in the following experiments was originally obtained from D'Herelle, a bacteriolysant antagonistic to shiga dysentery. Two c.c. of our stock bacteriolysant known as "31 A" was used in a 1:10 dilution. Stock 31 A on titration was active in about a 1:6,000,000,000 dilution against shiga dysentery.

#### I. FIRST SERIES. SET A

To 1 c.c. of the 1:10 dilution of stock 31 A were added 49 c.c. of glucose blood broth. The media was prepared by adding glucose broth to whole blood which was allowed to stand for several hours in the cold room and the supernatant fluid taken off. The  $p_H$  concentration was 8.2.

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. diluted bacteriolysant inhibiting shiga dysentery	1	2	3	4	5	6
5 c.c. bacteriolysant as controls	9	10				
5 c.c. Glucose blood broth	7	8				

To Tubes 1 and 7 were added three drops of an eight hour blood broth culture of *Meningococcus*. Three drop serial transfers were then made from Tube 1 through Tube 6. Tube 8 was kept as a broth control and Tubes 9 and 10 as bacteriolysant or lytic principal (LP) controls.

The entire series was then incubated at 38 degrees.

Results after incubation for 24 hours:—Tubes 1 to 6 inclusive distinctly cloudy.

Tube 7 cloudy.

Tubes 8, 9 and 10 clear.

48 hours:—Tubes 1 to 7 markedly cloudy.

Tubes 8, 9 and 10 clear.

Entire series of 10 tubes were filtered through a Berkefeld after 60 hours.

#### Second series:

The filtrate from the first series was then incubated 24 hours. It remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP*	1	2	3	4	5
5 c.c. GBB*	6	7	8	9	10
5 c.c. GBB	11				
10 and 15 c.c. LP	12	13			

\*LP—Lytic Principal.

GBB—Glucose Blood Broth.

To Tubes 1 and 6 were added three drops of an eight hour glucose blood broth culture of *Meningococcus*.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 11 served as a broth control and Tubes 12 and 13 as lytic principal controls. The series was then incubated at 38 degrees.

Results after incubation for 24 hours: Tubes 1 to 5 inclusive were cloudy but not so much as Tubes 6 to 10.

Tubes 6 to 10 very cloudy.

Tubes 11, 12 and 13 remained clear.

36 hours: Practically the same except the degree of cloudiness was more marked though the relative turbidity remained between the LP tubes and broth tubes.

Control tubes remained clear.

At the end of 48 hours the entire series was filtered through a Berkefeld and 10 c.c. of fresh GBB of same  $p_H$  concentration were added to the filtrate.

*Third series:*

The filtrate from the second series was incubated 24 hours and remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP	1	2	3	4	5
5 c.c. GBB	6	7	8	9	10
5 c.c. GBB	11				
10, 10 and 15 c.c. LP	12	13	14		

To Tubes 1 and 6 were added three drops of an eight hour blood broth culture of *Meningococcus*.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 11 GBB control. Tubes 12, 13 and 14 LP controls.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 inclusive almost entirely clear.

Tubes 6 to 10 cloudy.

Control tubes clear.

24 hours: Tubes 1 to 5 slightly cloudy.  
Tubes 6 to 10 very cloudy.

Control tubes clear.

36 hours: Tubes 1 to 5 remained the same.

Some debris in bottom of tubes.

Tubes 6 to 10 markedly cloudy.

Control tubes remained clear.

At the end of 48 hours all tubes were filtered through a Berkefeld and 10 c.c. fresh GBB of  $p_H$  8.2 concentration were added to the filtrate.

*Fourth series:*

The filtrate from the third series was incubated for 24 hours. It remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP	1	2	3	4	5
5 c.c. GBB	6	7	8	9	10
5 c.c. GBB	11				
15 c.c. LP	12	13	14		

To Tubes 1 and 6 were added three drops of an eight hour blood broth culture of *Meningococcus*.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 11 GBB control. Tubes 12, 13 and 14 LP controls.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 remained clear.  
Tubes 6 to 10 were cloudy.

Control tubes clear.

24 hours: Tubes 1 to 5 clear.

Tubes 6 to 10 cloudy.

Control tubes clear.

36 hours: Tubes 1 to 5 clear.

Tubes 6 to 10 very cloudy.

Control tubes clear.

48 hours: Tubes 1 to 5 clear.

Tubes 6 to 10 cloudy (markedly).

Control tubes clear.

72 hours: Readings the same.

At the end of 72 hours the series was filtered through a Berkefeld. During the above experiments of Set A, Set B had been built up in precisely the same manner. The remaining 1 c.c. of the 1:10 dilution of stock 31A was diluted with 49 c.c. of GBB of  $p_H$  8.2 and this material was carried through



the fourth series with meningococcus the same as Set A, excepting 20 c.c. was carried along with shiga dysentery. The results with Set B were exactly the same as with Set A and complete inhibition of growth of the meningococcus did not occur until the fourth series was reached.

At this point filtrates of Set A and B were combined. The combined filtrates were then carried through the 5th, 6th, 7th, 8th, 9th and 10th series with meningococcus with complete inhibition of growth in the LP tubes attending each series. When cultures to which a few drops of the adapted lytic principle had been added were plated out, there were found many transparent areas in which no cocci grew and if one of these transparent areas was touched with a sterile platinum loop and then drawn across the surface of a 12 and 24 hour blood agar culture of meningococcus, a clear streak developed in about eight hours. A titration at this point, with the already extensively diluted Stock 31 A, showed inhibition in dilutions of 1:500,000 to 1:800,000 with six, eight and ten hour cultures of the organism. Forty c.c. fresh glucose blood broth were added to the combined filtrates.

## II. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH BROTH CULTURES OF STAPHYLOCOCCUS ALBUS

The combined filtrates of Set A and B were incubated for 48 hours and remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5
5 c.c. GBB	6	7	8	9	10
5 c.c. LP	11				
5 c.c. GBB	12				

To tubes 1 and 6 were added three drops of a six hour culture (GBB) of *Staphylococcus albus*.

Three drop serial transfers were then made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 12 served as GBB control while Tube 11 served as LP control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 clear.

Tubes 6 to 10 cloudy.

Control tubes clear.

24 hours: The same except Tubes 6 to 10 were more cloudy.

36 hours: The same except Tubes 6 to 10 were more cloudy.

48 hours: The same. Tubes 6 to 10 markedly cloudy.

60 hours: The same. LP tubes clear.

72 hours: LP tubes remained clear.

## III. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF STAPHYLOCOCCUS AUREUS

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	12
5 c.c. GBB	6	7	8	9	10	11

To Tubes 1 and 6 were added three drops of a six hour GBB culture of *Staphylococcus aureus*.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 12 LP control. Tube 11 GBB control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 clear.

Tubes 6 to 10 cloudy.

Control tubes clear.

24 hours: Tubes 1 to 5 clear.  
Tubes 6 to 10 very cloudy.  
Control tubes clear.  
36 hours: Reading the same except Tubes  
6 to 10 were more cloudy.  
72 hours: Tubes 1 to 5 remained clear.  
Tubes 6 to 10 markedly cloudy.  
Control tubes clear.

#### IV. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF STAPHYLOCOCCUS CITREUS

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	11
5 c.c. GBB	6	7	8	9	10	12

To Tubes 1 and 6 were added three drops of a six hour GBB culture of Staphylococcus citreus.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 GBB control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 remained clear.  
Tubes 6 to 10 cloudy.  
Control tubes clear.  
24 hours: Tubes 1 to 5 clear.  
Tubes 6 to 10 very cloudy.  
Control tubes clear.  
48 hours: Same except Tubes 6 to 10  
more cloudy.  
72 hours: Tubes 1 to 5 clear.  
Tubes 6 to 10 markedly cloudy.  
Control tubes clear.

#### V. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF MICROCOCCUS TETRAGENOUS

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	11
5 c.c. GBB	6	7	8	9	10	12

To Tubes 1 and 6 were added three drops of a six hour GBB culture of Micrococcus tetragenous.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 GBB control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 clear.  
Tubes 6 to 10 cloudy.  
Control tubes clear.  
48 hours: Tubes 1 to 5 clear.  
Tubes 6 to 10 very cloudy.  
Control tubes clear.  
72 hours: Tubes 1 to 5 clear.  
Tubes 6 to 10 markedly cloudy.  
Control tubes clear.

#### VI. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF PNEUMOCOCCI. GROUPS 1, 2 AND 3. INASMUCH AS THE RESULTS WERE THE SAME FOR THE DIFFERENT TYPES THE RESULTS ARE GIVEN UNDER ONE GENERAL HEADING

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	11
5 c.c. GBB	6	7	8	9	10	12

To tubes 1 and 6 were added three drops of an eight hour GBB culture of pneumococci.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 GBB control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 clear.  
 Tubes 6 to 10 almost clear.  
 Control tubes clear.

24 hours: Tubes 1 to 5 clear.  
 Tubes 6 to 10 definite cloud.  
 Control tubes clear.

48 hours: Tubes 1 to 5 clear.  
 Tubes 6 to 10 very cloudy.  
 Control tubes clear.

72 hours: Tubes 1 to 5 clear.  
 Tubes 6 to 10 markedly cloudy.  
 Control tubes clear.

#### VII. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF HEMOLYTIC STREPTOCOCCI

This strain was exceedingly virulent and was obtained from a fatal case of infection in the University Hospital. The first results obtained with this organism using serum broth media were not dependable due to the appearance of a heavy precipitate in the inoculated tubes presumed to be globulin.

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	11
5 c.c. GBB	6	7	8	9	10	12

To Tubes 1 and 6 were added three drops of a ten hour GBB culture of hemolytic streptococci.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 GBB control.

Results after incubation at 38 degrees for 12 hours: All tubes clear.

24 hours: All tubes except 6 and 7 were clear. 6 and 7 slightly cloudy.

48 hours: Tubes 1 to 5 clear.  
 Tubes 6 to 10 cloudy.  
 Control tubes clear.

72 hours: Tubes 1 to 5 clear.  
 Tubes 6 to 10 markedly cloudy.  
 Control tubes clear.

VIII. The question arose as to whether the results obtained in the preceding experiments could be obtained direct with the bacteriolysant which was being carried along on shiga dysentery, that part of the original dilution of the 1:10 dilution of stock 31A saved from our Set B. These 20 c.c. had been carried along with shiga dysentery through several series and new broth had been added from time to time and complete inhibition was being obtained with the organism. Consequently this bacteriolysant was tried with the organisms used in the above experiments. The results were uniformly the same, showing absolutely no inhibition of growth of the organism during the first and second series. For example we give below the results obtained with staphylococcus aureus.

The filtrate was incubated for 24 hours and remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP inhibiting shiga dysentery	1	2	3	4	5	11
5 c.c. Glucose broth	6	7	8	9	10	12

To Tubes 1 and 6 were added three drops of a six hour broth culture of Staphylococcus aureus.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 broth control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 cloudy.  
 Tubes 6 to 10 cloudy.  
 Control tubes clear.

24 hours: All cloudy except control tubes.

48 hours: All cloudy except control tubes.  
 No inhibition in any of the LP tubes.

Identical results were obtained as above with micrococcus tetragenous, staphylococcus citreus, pneumococci and hemolytic streptococcus. There appeared to be a slight amount of inhibition in the first series with staphylococcus albus and there was complete inhibition in the second series.

#### CONCLUSIONS

1. It is possible to bring about adaptation of a bacteriolysant antagonistic to shiga dysentery to the meningococcus and other cocci.
2. This adaptation might be termed a "group" adaptation since by adapting the bacteriolysant to one coccus it was found also to have been adapted to other cocci.
3. These experiments suggest that there is only one species of bacteriophage and this can acquire activity against any organism.
4. A staphylococcus bacteriophage or a coccus bacteriophage developed by adaptation can be readapted to its original organism, as in this case shiga dysentery.
5. The possibility of a polyvalent bacteriophage adapted to a large number of organisms and inhibiting the growth of all at the same time.
6. The reaction of organisms to Gram stain is apparently not a factor in "bacteriophagy" as shown by adaptation to the pneumococci, micrococcus tetragenous, streptococcus and staphylococci of the gram-positive group and the meningococcus of the gram-negative group of organisms.



## THE BIOLOGICAL ASSAY OF PITUITARY EXTRACT\*

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### I. INTRODUCTION

IN the case of certain widely used drugs, notably the digitalis group, can-nabis, suprarenal extract and pituitary extract, exact methods of chemical assay are not at the present time available. For these drugs the so-called "biological assay" methods are in use. It is recognized of course that quantitative chemical methods are the goal to be sought, but lacking these, it is necessary to have in the meanwhile some way of determining the relative strengths, so that the preparations which are made available to the physician may be uniform. For most of the drugs mentioned above there have been devised more or less satisfactory methods, as shown by the fact that the preliminary reports of the various committees working on the question of revision of methods for the next edition of the United States Pharmacopeia show little inclination to change other than minor details of the present methods. The assay process of pituitary extract, *Liquor Hypophysis* of the U.S.P. IX, has however been subjected to a number of criticisms on many scores.

The necessity for some agreement as to method of assay is clearly shown by the facts indicated in Table I, which gives in comparative form the strength of ten of the preparations available to the physicians of the United States. Of these, five purport to be of the strength indicated in the U.S.P. IX, and practically all of them carry the statement "physiologically standardized." If the use of physiological assay methods and a Pharmacopeial standard results in the manufacture and marketing of preparations differing as widely in strength as these do, then clearly something is wrong. As a matter of fact the terms as thus used mean one thing to the manufacturer and to the physician another. To the maker they are a statement that the successive lots of the extract which he puts on the market from time to time are uniform in strength. In justice to the manufacturers it should be said that in the writer's experience this has always been found to be the case. To the physician on the other hand there is at least the inference that a given preparation is uniform in strength with any other preparation for which similar claims are made. He is usually quite surprised to discover that this is not actually so. This difference in strengths is a potential and an actual source of danger to his patients. Suppose for example that an obstetrician

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From the Pharmacology Laboratory of the University of Michigan Medical School, Ann Arbor. The investigation herein reported was initiated in 1920 at the suggestion of the Chairman of the Sub-Committee on Biological Assays of the Revision Committee of the United States Pharmacopeia. After the work was well along toward completion it was learned that Dr. H. H. Dale of the Medical Research Council of the British Medical Association was carrying out a similar study. His report which is very complete and thorough was most generously made available in the press proof.

has been in the habit of using the preparation given as Number 9 in this table, and for some reason he is unable to get this preparation with which he is perfectly familiar and the dose of which he has worked out by experience. He uses instead Number 2, which so far as the literature attached indicates, is exactly the same, being "physiologically standardized" and of the U.S.P. strength. Actually it is at least three times the strength of the preparation to which he has become accustomed. The possibilities of accident are sufficiently obvious.

After some thought the conclusion has been reached that it would be unwise to give the names of the preparations whose strength is given above. The interpretation of the facts is sufficiently clear, namely that a method of assay and suitable standards should be adopted so as to insure the marketing of extracts which are of a uniform strength. If the names of the preparations are given, the first conclusion to which a casual reader might come is that the stronger preparations are the better ones. This is by no means the case. In the first place, the correct dosages of pituitary extract have not

TABLE I

GIVING THE RESULTS OF ASSAYS OF TEN COMMERCIAL PREPARATIONS OF PITUITARY EXTRACT  
IN TERMS OF ONE OF THEM, NUMBER 1.  
RESULTS OF ASSAY

NUMBER OF PREPARATION	OXYTOCIC METHOD	PRESSOR METHOD
1.	10	10
2. U. S. P.	-	10 plus
3.	7-8	10 plus
4.	6	8-9
5.	6	6
6. U. S. P.	5-6	5-7
7.	-	5
8. U. S. P.	3	4-5
9. U. S. P.	3	3-5
10. U. S. P.	1-	2

been worked out, in the very nature of the case cannot be worked out, until there is a greater agreement among the preparations, but there are in the literature a large number of cases of untoward events resulting from the administration of too large amounts of the extract, or of its improper use.<sup>1</sup> It is the teaching of a number of the obstetricians that the doses used should be reduced. The manufacturers themselves have recognized this, and in some cases have reduced the strength of their preparations, or have put on the market solutions of lower concentration, marked for obstetrical use. But even under these circumstances accidents have happened. Under such conditions the best preparation for any physician is not the strongest, but the one with which he is most familiar. It makes no difference to him whether the preparation he uses is of a strength to rank in the first place or the fifth place in such a table, so long as it is strong enough to accomplish what he desires, and is uniform from sample to sample. His protection at present is to know one preparation and to stick to it. This is not the intent of the Pharmacopœia. In the case of other potent drugs, the official preparations conform to a definite strength, regardless of the manufacturer. It should be thus with

Liquor Hypophysis. Of course it does not lie in the province of the pharmacology laboratory or of the United States Pharmacopeia to say what dose the clinician shall use but just as obviously is it necessary that some procedure shall be adopted which will insure that the physician shall be able to buy pituitary extract anywhere on the open market and be assured that the preparation obtained corresponds in activity to those to which he has been accustomed.

In the second place, some of these extracts purport to be 20 per cent extracts, in terms of the fresh moist gland, some are 10 per cent, and in some cases the strength is not stated. The theoretical strength, when given, does not always correspond to that actually found in laboratory examinations. From the manufacturer's viewpoint, a 10 per cent extract which ranks fifth is a better preparation than a 20 per cent preparation which ranks seventh. From his viewpoint that preparation which contains the maximum strength per unit of fresh gland material is the best, being the most economical. The clinician however is concerned with this point only insofar as it affects the cost to him of the finished product. If the extract is potent, the efficiency of the manufacturing process concerns him not at all.

The fundamental principle of the physiological assay is that it measures the amount of the substance examined required to bring about the same quantitative changes in the behavior of some physiological mechanism as is produced by a known amount of a standard substance having the same pharmacological action. It has generally been insisted that the assay method should be based upon the activity for which the drug is used in therapeutics. However, as Dale<sup>2</sup> has clearly pointed out, it is only necessary that the method measure in some way the activity of the same substance as that having the action for which the drug is used in its therapeutic applications. For example, in the case of the assay of pituitary extract, if it can be clearly shown that the constituent which produces the rise of blood pressure when injected into the veins of a laboratory animal is the same as that which causes contraction of the uterus in the pregnant woman, then it is perfectly permissible to use the laboratory determination of the pressor activity as an index of the oxytocic strength of the drug as used in the clinic. This point will be taken up again later. "Useful Drugs"<sup>3</sup> gives the following summary of the action of the extract of the posterior lobe of the pituitary gland, or the Liquor Hypophysis of the U.S.P. IX: "It stimulates the uterus to contract, whether intact or excised, it increases the blood pressure by peripheral constriction, stimulates the kidney to increased secretion of urine, causes broncho-constriction, stimulates the smooth muscle of the mammary gland \* \* \* and in certain pathological conditions at least stimulates the intestine and bladder to increased contraction." Of necessity one must take the measure of the physiological activity along one of these lines as an index of its therapeutic strength.

## II. THE PRESSOR METHOD OF ASSAY

The assays of Table I are made by two methods, the pressor method, that of comparing the doses of known and unknown extracts required to



produce equal changes in the blood pressure, and the oxytocic method, which consists in determining the doses of known and unknown having the same activity on the excised uterus of the virgin guinea pig. These two methods are the only ones which have received any serious attention in the standardization of pituitary extract.

The pressor action of the extract of the pituitary body was described by Oliver and Schäfer.<sup>4</sup> Howell<sup>5</sup> showed the action was confined to extracts of the posterior lobes. Hamilton<sup>6</sup> suggested the use of the pressor reaction in determining the strength of extracts. Roth<sup>7</sup> compared a number of commercial preparations by this method and in 1916 Hamilton and Rowe<sup>8</sup> reported in detail the method as applied to the standardization of one of the commercial extracts. The method has been used repeatedly in this laboratory, essentially as outlined by Hamilton and Rowe. Dogs are used, and chlore-tone dissolved in olive oil is given intraperitoneally. The vagi are cut to eliminate reflexes, and the blood pressure is taken from the carotid in the usual way. Alternate doses of standard and unknown are given at fifteen-minute intervals until a dose of the preparation being assayed is found giving the same pressure increases as the standard. The strengths of the two extracts are then in inverse proportion to the doses used. The results of assays carried out by this method are in general more or less parallel to those obtained by the isolated uterus method (Table I). The technic, while not difficult, requires more attention than the excised uterus method, so that it actually seems less tedious (Dale, 1922, with which the writer agrees).

There is one very good reason why such a test should not be adopted as an official method of assay for pituitary extract. It is quite certain that the most important use of pituitary extract is for its action on the uterus. If the pressor and oxytocic bodies were the same, it would be quite proper, as pointed out in a previous paragraph, to use the pressor action in the dog as a measure of the oxytocic action in the human species. But Dudley<sup>9</sup> has shown that it is possible to separate in part at least the oxytocic and pressor bodies, and in a later paper not yet published he gives evidence that there are probably *two* pressor substances as well as the substance which stimulates the uterus.<sup>10</sup> Under such circumstances it is quite illogical to use the pressor test to determine the oxytocic activity. In this connection it has been reported several times that the values for particular extracts as found by the two methods do not always run parallel.<sup>2, 7, 11</sup>

### III. THE OXYTOMIC METHOD OF ASSAY

The oxytocic action of extracts of the hypophysis was first pointed out by Dale in 1906.<sup>12</sup> He later gave a clear picture of the action and localized the point of action as being on smooth muscle.<sup>13</sup> Kehrer recommended the use of the isolated uterus as a test organ for determining the strength of ergot preparations.<sup>14</sup> Engeland and Kutscher used the isolated uterus of the cat as a means of determining the pharmacological action of the various fractions which they separated from pituitary extract.<sup>15</sup> It was Dale and Laidlaw,<sup>16</sup> however, who first definitely worked out a method for comparing quantitatively the strengths of pituitary extracts using the isolated uterus



of the guinea pig. They were led to the use of this organ by certain theoretical considerations. A method which is to give quantitative results must not be complicated by the question of tolerance. To put it differently, the amplitude of response of any physiologic mechanism to given doses of a drug must not be conditioned by the preceding dose. When pituitary extract is injected into the blood stream, there is an increase of blood pressure which is maintained over a relatively long period. The pressor substance is eliminated or destroyed rather slowly, so that the circulation returns to its normal condition quite gradually, and a certain degree of tolerance to succeeding doses remains. "It seemed quite probable that a thin strip of isolated muscle, suspended in a large volume of Ringer's solution, which could be rapidly removed and replaced by a fresh volume, would have a much better chance of recovering its original condition by the rapid washing out of the principle, or the reduction of its concentration below the threshold of activity." They found the isolated horn of the uterus of the young virgin guinea pig most satisfactory, as when suspended it assumes a condition of low tonus, broken only by occasional small contractions. As a standard they used a 10 or 20 per cent extract of fresh moist gland, sterilized in ampoules and kept in this form. It was clearly pointed out in the conclusion of their paper that the use of such a standard made the method essentially a comparative one, not giving absolute values. An absolute standard was not suggested because of the lack of a substance having an action qualitatively similar to that of pituitary extract. Roth<sup>17</sup> and Heidelberg, Pittenger, and Vanderkleed<sup>18</sup> verified the value of the method as developed by Dale and Laidlaw. Roth recommended the use of histamine as a standard against which to compare the unknown extracts, in place of the "standard extract" of previous workers. In 1915 the ninth revision of the United States Pharmacopeia appeared, incorporating Roth's suggestion and outlining the method which is official at the present time. The U.S.P. IX gives the following requirement for the Liquor Hypophysis: "One mil of Solution of Hypophysis, diluted 20,000 times, has the same activity on the isolated uterus of the virgin guinea pig as a 1 to 20,000,000 solution of beta-aminazoly-ethylamine hydrochloride when tested as directed by the United States Hygienic Laboratory." (As a matter of history it may be stated that this action was not taken by the Sub-Committee on Biological Assay, which explains why the method was not included in the section on "Biological Assay.")

The directions of the United States Hygienic Laboratory are those given by Roth.<sup>7</sup> Young virgin pigs weighing from 275 to 350 grams are recommended. A piece of uterus one to two centimeters long is used, and is weighted enough to overcome the tonus resulting from the necessary manipulation. A temperature of about 39° C. is used. Further details are given as to the arrangement of stock bottles, thermostat, etc., most of which details are modified in the various laboratories using the method.

Since this method was made official, a number of criticisms and suggestions have been made, chiefly on the part of the representatives of the various manufacturing houses, as they are the ones to use the test most extensively. In 1918 Spaeth<sup>19</sup> gave a very good summary of these objections, pointing

out that they fall into three classes: (1) objections as to the nature of the test; (2) objections to the use of histamine (beth-iminazoly-ethylamine) as a standard; (3) objections to the requirement as to strength of the finished commercial extract as given in the U.S.P.

(1) *Objections to the Nature of the Test.*—Other methods of assay have been suggested, as pointed out above, based on other physiologic properties of the extract. Dale and Laidlaw compared extracts as to their pressor action, and at that time concluded that for practical purposes the results were parallel to those obtained by the oxytocic method, but held that the latter method was more accurate, and more desirable on theoretical grounds. At the present time Dr. Dale does not hold to this view, and reports that he has examined preparations in which the oxytocic and pressor activities were not parallel.<sup>2</sup> Hamilton<sup>6</sup> and Hamilton and Rowe<sup>8</sup> consider that the pressor method gives a more accurate picture of the therapeutic strength of the extract than does the oxytocic test. They point out that histamine, against which the U.S.P. requires that Liquor Hypophysis be tested, has no value in obstetrics, and ask whether it may not be merely a coincidence that pituitary extract acts to stimulate the excised uterus and the gravid uterus as well. As a matter of fact histamine acts on the uterus *in situ* but has other systemic actions which make its use in obstetrics impracticable. The fundamental objection to the use of the pressor method has been pointed out in an earlier paragraph.

Under the heading of objections to the nature of the test, Spaeth groups also the statements of technical difficulties involved. These have been met to a greater or less degree by all who have had occasion to use the method. The greatest difficulty is that of obtaining satisfactory uteri. Roth recommends those from virgin guinea pigs of a weight of from 275 to 350 grams. Uteri from animals which are or have recently been pregnant, or are in heat, are discarded. The ideal uterus is one which when suspended in the bath of Ringer's soon reaches a condition of minimum tonus upon which are superposed a succession of contractions of mild intensity. When the proper amount of pituitary extract is added to the bath the tonus increases fairly rapidly and quite regularly. (Fig. 1.) The amplitude of tonus increase is in a general way proportional to the concentration of the extract. If the concentrations are kept within certain limits, the contractions are submaximal, and when the pituitary extract is washed off there is a prompt return to the original low level. However, some uteri are found which do not give this satisfactory picture. Some are hyperirritable, giving maximal contractions to all effective concentrations. Others give such large spontaneous contractions that it is difficult or impossible to differentiate between the contraction due to the drug action and that due to the spontaneous action. These difficulties may at times be avoided by increasing the tension against which the uterus has to contract, but again, this procedure may and often does result in a decrease in the irritability of the strip, making it useless for the test. This means a loss of one or two hours in experimenting with an unsatisfactory strip. Another difficulty, not so frequent of occurrence, but more serious, is the obtaining of strips which will not give to two successive applications of the same concentration of pituitary extract, the same ampli-

tude of contraction. Pittenger<sup>20</sup> states that this condition can often be remedied by increasing the weights exerting tension on the uterine strip.

These difficulties have been encountered by every worker, and are mentioned in practically every paper dealing with the use of guinea pig uteri for assay purposes. Various individuals have attempted to get around the trouble by trying to learn some method of selecting suitable animals before sacrificing them. Practically all workers are agreed that the larger animals, weighing above 300 or at most 350 grams, and those pregnant or in heat, should be rejected. Trendelenberg and Borgmann<sup>21</sup> have studied this question in some detail, and insist that only those uteri which are obtained from small pigs, and which give very small spontaneous contractions, should be used. They maintain that with the uteri from larger pigs the amplitude of the contraction does not of necessity follow parallel to the concentration of pituitary extract, but that often increasing the concentration acts to shorten the latent period rather than to increase the amplitude of contraction. They do not use animals weighing over 250 grams.

The most recent statement on the matter is in the paper by Burn and Dale.<sup>2</sup> They find that if young females are kept out of sight and smell of the males, from the time they are weaned, and are used when weighing from 200 to 300 grams, and moreover are not used during heat, that practically all of these difficulties may be avoided. The experience of the writer confirms this, as does that of Eckler (personal communication).

Of the above writers, all of whom admit to a greater or less degree the difficulties to be met in applying the oxytocic test, only Hamilton and Rowe state that it should not be retained as official.

Kochmann<sup>22</sup> has recently described a method of getting around the difficulties offered by hyperirritable uteri, and at the same time he attempts to make possible the utilization of the larger sizes of guinea pigs which may be available. He reduces the irritability of the uteri by reducing the calcium content of the Ringer-Locke, and if necessary by adding magnesium chloride to the solution, before treating the uterus with pituitary extract. Histamine is used as a standard. The sequence of application of successive doses is entirely different from that ordinarily used, and he utterly ignores the changes in irritability which occur spontaneously in such preparations. The method has not been tried in this laboratory.

Spaeth<sup>23</sup> realizing the difficulty of the uterine method, suggested a more objective method, i.e., that of comparing the extracts to be standardized by measuring the degrees of contraction which they produced in the melanophores of the isolated scales of a fish. The method, so far as known, has not been tried by other pharmacologists, who seem for some reason to feel that it represents an excursion too far into the fields of the zoologist. It is of course open to the objection pointed out by Dale for the pressor method of assay, in that it is not proved that the substance which causes the melanophores to contract is the same as that which causes the uterus to contract.

(2) *Objections to the Substance Used as a Standard.*—There have been many objections to the use of histamine as a standard substance. These have been well summarized by Spaeth.<sup>19</sup> The objections can be placed in two main



groups; (a) those concerned with the uniformity of the various specimens of the salt and the stability of the salt or its solution; (b) those concerned with the qualitative similarity or dissimilarity to pituitary extract with respect to its action on the excised uterus.

(a) Roth<sup>17</sup> investigated the physiologic activity of the three samples of histamine available to him before recommending its use as a standard, and found them to be uniform. He also found that solutions did not lose in activity when autoclaved in ampoules on three successive days. Pittenger and Vanderkleed<sup>24</sup> however reported that the dry salt did not keep. They prepared solutions which were set aside for a year. At the end of that time fresh solutions of the same theoretical strength were prepared from the same stock of crystals. The year old solutions were stronger than those made up freshly. This they interpret as meaning a deterioration of the dry salt. They do not mention the possibility of the salt being deliquescent. If this were the case, of course the new solutions would be weaker than the old. Spaeth<sup>19</sup> agrees with the conclusions of these authors. The samples of histamine available to him did not agree either in their physiologic strength or their physical constants. He pointed out that the salt is hygroscopic, which introduces the possibility of a very considerable error in weighing. These considerations precluded its use as a standard and he recommended as a substitute the use of potassium chloride.

It had previously been shown by this author that the melanophores of fish scales responded in a quantitative manner to potassium chloride solutions and inasmuch as he believes the melanophores to be smooth muscle cells much modified, he argued that the smooth muscle of the uterus ought to behave in a similar manner. Experimental work seemed to bear out his argument and in 1917 he recommended the substitution of potassium chloride as a standard substance, suggesting that in order to make this standard conform to U.S.P. requirements, a commercial preparation should have in a 1:4,000 dilution the same effect on the isolated uterus as a 0.14 per cent solution of potassium chloride in Ringer's solution.

(b) There are various hints in the literature that the action of pituitary extract and histamine are not exactly parallel, but no evidence given until the recent paper by Burn and Dale.<sup>2</sup> This of course would entirely preclude the use of histamine as a standard.

(3) *Objections to the Strength of the Present Requirement.*—Few if any of the preparations in use at the present time actually correspond in strength to the standard given in the U.S.P. IX. They are instead generally from three to ten times this strength. For example, the chemist of a manufacturing concern writes that in his laboratory it is the practice to consider an extract as satisfactory when in a dilution of 1:200,000 it has the same activity on the isolated uterus of the guinea pig as a 1:20,000 solution of histamine hydrochloride. In other words his preparation is just ten times the official strength. Whether this preparation is too strong is another question, but at any rate it is the view of the writer that the majority of the preparations now sold, and with which this generation of physicians is familiar, are far stronger than the Liquor Hypophysis of the present Phar-



maeopeia, and that in formulating a new standard, the strength should be set so as to approximate as nearly as possible the average of accepted commercial practice, which after all gives at least an indication of the requirements of the medical practice. If the profession can be supplied with a uniform preparation, then it will be in a position to work out the proper doses for the various clinical indications.

#### IV. EXPERIMENTAL

(1) *Potassium Chloride as a Standard.*—It seemed worth while to investigate the possibility of the use of potassium chloride as a standard, since it seemed to meet clearly the requirements set forth by Spaeth as desirable in a standard, namely (1) qualitative similarity to pituitary extract with respect to its smooth muscle action; (2) parallelism in the changes in irritability of uterine strips to the extract and the standard substance (this was emphasized by Spaeth as being of most fundamental importance); (3) stability and ease of obtaining in pure form; (4) relatively high concentration required to affect the uterus. He pointed out in connection with this last point that the chance of error in dilution of solutions of a substance such as histamine is extremely large, since histamine is active in very dilute solutions. Since potassium chloride seemed to meet all of the above requirements, a number of standardizations were carried out with its use.

The technic followed was essentially that used in every laboratory where the activity of smooth muscle is studied. The apparatus consisted of a tube with a capacity of 100 c.c., in which the uterus was suspended. This tube was surrounded by several liters of water in a copper bath, kept at constant temperature by a small gas burner, regulated by a thermostat. The tube was arranged so that the waste fluids could be drawn off at the bottom, and fresh Ringer's added in the same way. This fresh solution was run through a large glass coil having a capacity of something over 100 c.c., which was placed in the water-bath. The solution from the stock bottle was siphoned through this coil, and was thus heated to the same temperature as the fluid in the tube surrounding the uterus. This arrangement makes it possible to have a constant supply of fresh Ringer's at the proper temperature and at the same time does not keep any more of the stock heated than is immediately necessary. Eckler has described an apparatus which is quite similar.<sup>25</sup> The required amounts of pituitary extract and standards, whether potassium chloride or histamine were blown from pipettes into the tube. The accuracy of dilution obtained by this method of addition and dilution in the tube is of course not so great as that described by Roth,<sup>7</sup> in which the proper dilution is made up outside the muscle chamber, and then used to replace the Ringer's in which the muscle has been suspended. However, it has been felt that the possible errors of dilution by the method as used in this laboratory have been well within the limits of accuracy of comparisons, and in making the dilutions in this manner, the stimulus of exposing the muscle to the air momentarily has been avoided. The solution in which the muscle is suspended is aerated from a tank of compressed oxygen.

The Ringer-Locke used in the earlier comparisons was of the following composition:

NaCl	.....	0.9%
CaCl <sub>2</sub>	.....	0.026%
KCl	.....	0.042%
NaHCO <sub>3</sub>	.....	0.01%

Later the composition was changed to agree with the formula given by Burn and Dale,<sup>2</sup> in which the bicarbonate is increased to 0.05 per cent, dextrose is added to make 0.05 per cent, and magnesium chloride 0.0005 per cent. This solution has proved more satisfactory, and is now used in all isolated uterus work. It should be emphasized that the salts used must be of the highest purity obtainable. The strength of the final dilution of the potassium chloride standard solution was that recommended by Spaeth, 0.14 per cent.

At first the results of the experiments with the use of this method

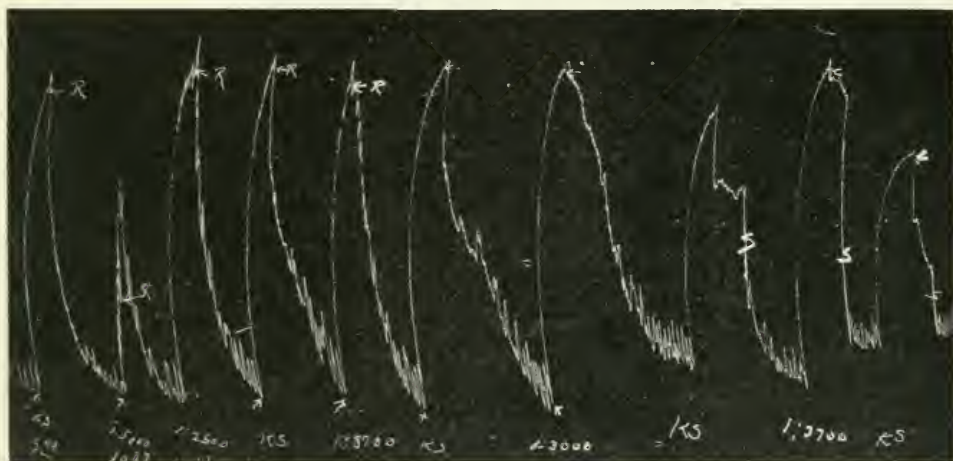


Fig. 1.—The lack of parallelism between the changes in irritability toward potassium chloride as compared with pituitary extract. KS = 0.14% KCl, the standard of Spaeth. At the beginning pituitary extract in 1:2500 dilution is equal to KS. A dilution of 1:3700 is too weak, but the second application of 1:3700 is too strong. There is an interval of 97 minutes between the two applications of 1:3700. At S and S' the drum was stopped.

were quite promising, so much so that at the meeting of the Revision Committee in Philadelphia in 1920, a preliminary report was made in which the use of the KCl standard was recommended. Further experience however has shown that the substance is unsuitable. The results of a number of experiments are given here to show the basis for the objections to potassium chloride. Fig. 1 shows the curve from an experiment in which the changes in irritability to potassium chloride and pituitary extract are not parallel. At the beginning of the experiment a 1:2,500 dilution of pituitary extract is the equivalent of 0.14 per cent KCl, and a 1:3,700 dilution is clearly inferior to the KCl standard. After an interval of fifty minutes, a 1:3,700 dilution is just as clearly too strong. In other words the changes in irritability are not parallel. Either the irritability of the strip to potassium chloride is reduced, or else it is increased to pituitary extract. It

might be mentioned in passing that this particular preparation of pituitary extract was by far the weakest examined. It is Number 10 of Table I. In Fig. 2 are shown the results of a comparison of potassium chloride and pituitary extract on the two horns of a uterus. The first comparison (*a*) was carried out immediately on removal of the uterus, while (*b*) was made twenty-two hours later, the second horn having been kept in the meanwhile on ice. The pituitary extract used was diluted the first day and kept in the interval in the cold. In the tracing from (*a*) to 1:5,000 dilution of pituitary extract is about the concentration required to equal the standard. Clearly 1:6,000 is too weak, and 1:4,000 too strong. In the second part,

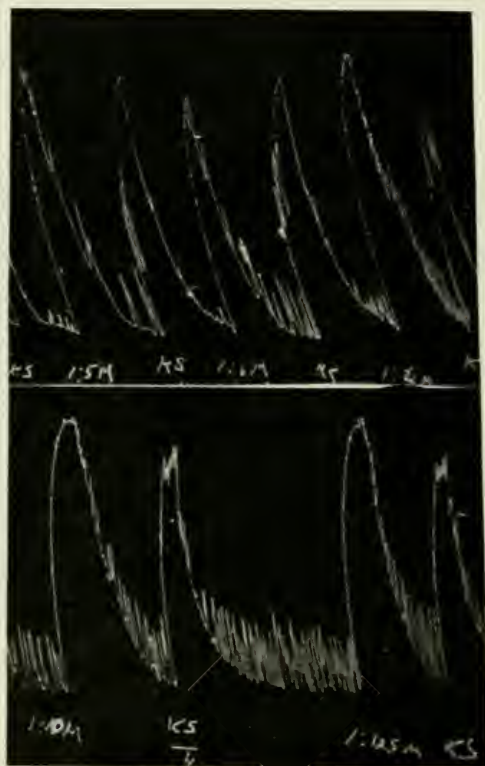


Fig. 2.—The different response of two horns of the same uterus to the potassium chloride standard. *A* was used immediately on removal from the body. *B* was kept on ice 22 hours. The same pituitary extract was used on both strips. In *a* the KCl standard is clearly stronger than pituitary extract, 1:6000 (6M), while in *b* it is just as clearly weaker than 1:12,500 (12.5M).

1:12,500 is clearly too strong. It was later found that at least at the end of the experiment 1:20,000 was about correct. Such an experiment merely exaggerates the differences in the changes in irritability toward the two substances, but it offers additional evidence that the two substances do not act in a qualitatively similar manner on the excised uterus. The changes differ in magnitude but not in kind from those shown in Fig. 1.

Another objection to the use of potassium chloride as a standard is that the strips subjected to its influence do not retain their activity so long as those subjected merely to the action of pituitary extract. This is a



conviction based on the impression obtained from a large number of standardizations in which potassium chloride was used, rather than on the results of experiments planned to test the point. It is of course of little importance as compared with the more serious objection of the lack of parallelism in the changes in irritability toward the two substances used. It is not intended to imply that the types of responses just discussed occurred in every instance. As a matter of fact the first difficulty with this method came from an entirely different source. The first dilutions of pituitary extract used were those given in the Hygienic Laboratory Bulletin No. 115,<sup>19</sup> which proved to be entirely too strong, and it took some time to discover that it was necessary to use very much more dilute solutions in order to avoid giving concentrations which produced maximal contractions. It is hard to understand the concentrations given by Spaeth. It has been necessary to use dilutions twenty to fifty times greater than his in order to avoid maximal contractions. The particular extract studied in the experiment from which Fig. 1 is taken was the only one which came anywhere near his recommendation, namely that a satisfactory extract should correspond to the 0.14 per cent potassium chloride standard when used in a 1:4,000 dilution.

It is felt that the considerations as given, especially the lack of parallelism in changes in irritability toward the two substances, entirely preclude the use of potassium chloride as a standard substance in the assay of pituitary extract.

(2) *Histamine as a Standard*.—The standard adopted by the U.S.P. IX for pituitary extract is of course histamine hydrochloride. As pointed out in the discussion of the literature, a large number of objections have been raised to the use of this substance, these being concerned with the stability of the salt and the qualitative similarity of its action on the uterus to that of pituitary extract. The impression among most of the pharmacologists seems to be that it is quite stable under ordinary conditions. For example Dudley<sup>9</sup> has shown that a solution of histamine phosphate is not affected by standing at room temperature (17° C.) for two hours with an equal amount of 2N sodium hydroxide. However, Pittenger and Vanderkleed<sup>24</sup> seemed to offer some proof of its deterioration, and Spaeth held the same view. Roth,<sup>17</sup> at the time of making the first recommendation as to its use, offered evidence that it did not lose in activity in the practical use of the substance in making a number of assays. At the meeting of the Society of Pharmacology and Experimental Therapeutics at New Haven in 1921, most of the pharmacologists there present seemed to question the lack of stability. Certain experiments have been carried out here which seem capable of interpretation only on the ground of instability.

The histamine used was the hydrochloride, "Imido" Roche, obtained from Hoffmann LaRoche Company, New York. On February 4, 1921, 50 mg. were weighed out and dissolved in 50 c.c. of freshly distilled water. One c.c. fractions of this solution were then placed in glass ampoules which were sealed and sterilized by boiling. These ampoules were kept in the dark, and were used as the stock from which fresh dilutions were made up as needed. In January, 1922, 20 mg. of the same specimen of histamine as used in 1921,



and dissolved in 20 c.c. of distilled water. This fresh solution was compared with the ampoules of a year earlier. The results are shown in Fig. 3. They clearly indicate that the new solution is distinctly weaker than the old. This result is capable of two explanations, either the crystals had taken up water, or they had lost in activity. The stock of crystals had been kept during this interval in a tightly stoppered glass bottle, which was not opened during that time.

The new solution was then placed in ampoules, and part of it sterilized by boiling on three successive days. It was then compared with the unboiled new solution. The results are given in Fig. 4. Here again, the boiled solution is weaker than the unsterilized solution. There seems to be no explanation except that there is a loss of activity in the boiling. The results have been checked to eliminate the possibility of errors of dilution.



Fig. 3.—Experiment to show the difference between a fresh solution of histamine, and a solution of the same stock which had been kept a year. Solution "O" was made up February 4, 1921, in 1:1000 strength, and sterilized by boiling in glass ampoules for three successive days. "N" was made up in the same strength January 23, 1922, and examined without sterilization. The new solution is clearly inferior to the old in strength.

When the stock of crystals was examined in September of 1922, it was clearly seen that they had taken up some water. Whether the loss of activity on boiling can be corroborated by other workers or not, the hygroscopic action of this particular salt of histamine should preclude its use as a standard.

The other group of objections to the use of histamine as a standard are those which have to do with the qualitative similarity in the action of histamine and pituitary extract. With the exception of the recent paper of Burn and Dale<sup>2</sup> there have been no definite statements on this point, other than those of Roth,<sup>7</sup> who finds that the actions of the two substances are qualitatively similar. The Physiological Assay Committee of the American Pharmaceutical Association suggested that this point be investigated, being evidently somewhat skeptical on this score. Dr. Dale, in a personal communication, has shown tracings which bring out very clearly the fact that in

some cases there is a lack of parallelism in the changes in irritability, similar to that in the case of potassium chloride. The writer has had similar experiences, and in addition has had experiments in which it was possible to get a fairly good approximation on one strip of uterus, which did not agree with subsequent comparisons made on the other half of the same uterus. (Fig. 5.) In the first part of this experiment the amounts of the two solutions given to cause the same amplitude of contraction stand in the ratio of one to six. In the second part of the same experiment, carried out on the other horn of the same uterus, the ratios are of one to eight, one to six being clearly too strong. It should be stated that this stock solution of histamine was diluted to one part in fifty thousand, so that the final concentration applied to the uterus in the first experiment would be 1:16,666,000.

It seems to the writer that the lack of parallelism in the changes in irritability as between histamine and pituitary extract, the hygroscopic na-

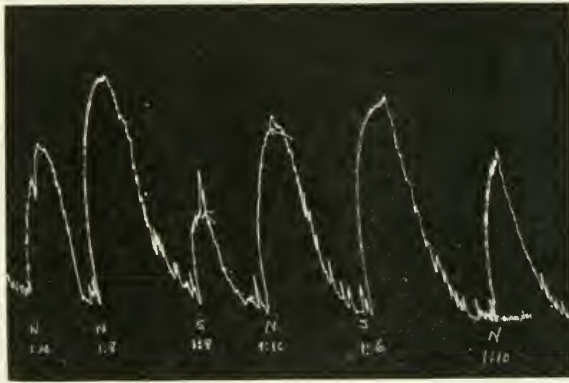


Fig. 4.—Experiment to show the loss of activity of histamine during the sterilization process. A solution of histamine was made up fresh, and placed in ampoules. Part of the ampoules were sterilized by boiling for twenty minutes on three successive days. The contents of one of these ampoules was then diluted and compared with a similar dilution of one of the same batch of ampoules which had not been subjected to the boiling process. *N*, not boiled; *S*, boiled. Dilutions in millions. Clearly *S* is weaker than *N*.

ture of the histamine, and the evidence as to instability under the conditions of use are enough to preclude the use of histamine as a standard.

#### V. DISCUSSION

It would seem that the lack of a substance having a qualitative similarity to pituitary extract in its action on the excised uterus, would make it necessary to use some preparation of the gland itself as a standard. This was of course the first standard employed, and in those laboratories in which the pressor method has been developed, it is still used. Others who actually have used histamine, have maintained that a pituitary preparation of some sort would be preferable. The first objection to such types of standards is of course that they do not make possible exact evaluations. This objection was pointed out by Dale and Laidlaw and later by Roth. It still constitutes a very serious objection, but in the absence of any better plan, it seems necessary to ignore it. After all, the purpose of an assay method is to insure the preparation of extracts of uniform potency for the medical profession. If a

pituitary preparation can be made which can be easily duplicated in any laboratory, without important variations in strength, then this preparation will meet the practical requirements of a standard, and also the theoretical one of qualitative similarity of action. The paper of Burn and Dale makes very definite recommendations as to the preparation of such a standard from the fresh pituitary glands. They have convinced themselves of the uniformity of the content of oxytocic substance from gland to gland, and are therefore using a preparation made from six or eight fresh glands by a rigidly pre-

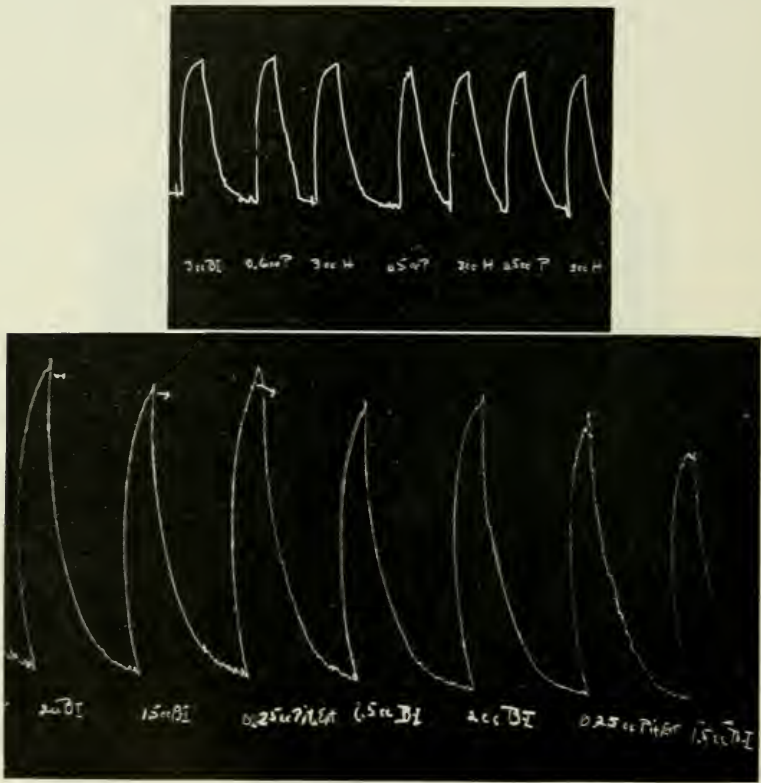


Fig. 5.—Comparison of histamine and a pituitary extract on the two horns of the same uterus. In *a* the amounts of the two substances added from the pipette stand in the ratio of one to six. In *b*, which was carried out about five hours after removal of the uterus from the pig, the ratio is one to eight, one to six being clearly too strong. The histamine solution was a 1:50,000, and the bath had a capacity of 100 c.c., so that 0.3 c.c. of histamine solution gives a concentration of 1:16,666,000.

scribed process. The data which they have presented seem convincing, but are being worked over in this laboratory with the cooperation of others concerned.

VI. SUMMARY

It has been shown that potassium chloride and histamine are not suitable substances for the standardization of pituitary extract. Since no other material which is yet available has an action qualitatively similar to that of pituitary extract, it is felt that a preparation of the gland itself should be used as a standard.

Because of the evidence given elsewhere as to the nonidentity of the oxytocic and pressor substances in the pituitary gland, the pressor method should not be employed in the assay of a drug which has its chief use for its oxytocic action.

It is expected that a definite recommendation will be made very shortly as to the details of preparation of a standard from the pituitary gland itself, together with the details of the exact method to be followed in the biological assay of pituitary extract.

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## LABORATORY METHODS

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### A NEW METHOD FOR THE DETERMINATION OF CALCIUM, MAGNESIUM, POTASSIUM AND SODIUM IN HUMAN BLOOD\*

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THE determination of calcium, magnesium, potassium and sodium has been carried out by several investigators. The usual method consists of getting rid of the protein, (either by oxidizing it with a mixture of concentrated sulphuric and nitric acids or by precipitating the protein with trichloroacetic acid) and determining calcium as oxalate, magnesium as magnesium-ammonium-phosphate or magnesium-ammonium-arsenate, and the total amount of potassium and sodium as sulphates; potassium as potassium-cobalt-nitrate or potassium-chloroplatinate, and sodium from the difference between the total amount of the alkali sulphates and the calculated amount of potassium sulphate or directly as sodium pyroantimonate.

This method is open to several objections. It has been pointed out by no less an authority than the official Association of the Agricultural Chemists that the cobalt-nitrite method for the determination of potassium yields inaccurate figures on account of variations in the formula of potassium-cobalt-nitrite. This objection has been disregarded by several investigators, who must have reasoned that the named variations are of little significance in the determination of such small quantities of potassium salts as are usually found in the blood.

The authors are in a position to state that the variations may affect considerably the accuracy of the results. The proof was furnished by determining the amount of potassium in an aqueous solution which contained all four above-named metals in the same quantities as they are usually met with in the blood. Platinum is too expensive to be used in analytical work and for that reason the chloroplatinic acid has been superseded by other reagents (perchloric acid, sodium-cobalt-nitrite).

The formula of either magnesium-ammonium-phosphate or magnesium-ammonium-arsenate is also subject to variations and should not be used for the determination of magnesium, although the perspective of determining magnesium colorimetrically which it offers (liberating, aerating and Nesslerizing the ammonia of magnesium-ammonium-phosphate or magnesium-ammonium-arsenate) is rather fascinating.

The determination of the inorganic constituents of the blood is still in

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the macrochemical stage. We do not yet possess such methods for the determination of calcium, magnesium, etc., which would rival in delicacy those used for the determination of urea, creatinin, uric acid or sugar. The quantity of calcium and magnesium usually found in the blood being very small, we have our doubts as to the possibility of obtaining reliable results with less than 25 c.c. of blood. This amount should be considered the minimum, and if larger amounts are obtainable, the better.

We must not forget that little is yet known as to the rôle of such metals as calcium and magnesium in the human body, and therefore, the more reliable the results of analysis, the better the cause of the knowledge is served. We have plenty of hypotheses with the main weight resting on speculations and fanciful assumptions instead of reliable analytical data. Before the amount of the blood necessary for the determination of the inorganic constituents can be reduced, colorimetric methods should be devised which would equal in their accuracy the familiar blood chemistry methods.

The oxidation of blood with a mixture of concentrated sulphuric and nitric acid is anything but pleasant considering the large amount of the acid mixture which has to be evaporated. Yet, we are not sure whether the precipitation of the protein with trichloroacetic acid is correct since we are totally unaware of the amount of the metals which may be combined organically. We therefore modified the oxidation method so as to make it less objectionable and more convenient. We proceed as follows:

We add slowly and at short intervals about 75 c.c. of a mixture of 100 c.c. of fuming nitric acid and 15 c.c. of concentrated sulphuric acid. (Sp. Gr. 1.84.) After every addition there is a violent reaction with copious foaming. As a precaution against overflowing, we use a 1000 c.c. flask and wait after each addition a few minutes until the reaction subsides. It is quite safe to add 20 c.c. at a time, proceeding slowly, adding and shaking the flask while adding. Cooling is not necessary. After all the acid has been added, driving off of the latter is immediately started. This is easily accomplished when the flask in which the oxidation has been carried out has a ground tubulated (single tube) glass stopper with the outside part of the tube bent under a sharp angle, and the inside about  $\frac{1}{2}$ " long. The outside part of the tube is then led into a somewhat larger tube and the connection made air tight with a piece of rubber tubing. If the tube of the glass stopper reaches far enough into the larger tube (about 3"), there is no chance for the hot acid fumes to eat through the rubber. The larger tube passes through the hole of a double perforated cork stopper into a bottle containing 500 c.c. of water. The tube ends at least 2" above the surface of the liquid, care being taken that throughout the whole reaction it remains outside of the water. The tube which passes through the other hole is bent under a convenient angle and led outside. The same bottle can be used to absorb the excess acid from more than one flask where oxidation is going on, provided a flask with a sufficiently large mouth is chosen.

The concentration of the acid mixture is carried on in the same flask where the oxidation has taken place. The boiling off is stopped when the

volume of the liquid is no more than 5 or 6 c.c. The further treatment will be described later.

Briefly stated our method is as follows:

Calcium and magnesium are precipitated and weighed as stearates (more correctly as a mixture of stearates and palmitates). The latter are then dissolved in N/100 sulphuric acid, the precipitated stearic acid filtered off and the excess of the sulphuric acid titrated with N/100 sodium hydroxide. The total amount of the stearates and that of the stearic acid contained in both of them being known, the respective amounts of calcium and magnesium can be calculated.

We tried to avoid the re-dissolving of the metal bases by titrating the unchanged amount of ammonium stearate and thus determining the quantity of stearic acid which went into reaction. We found the end point to be too vague.

The metals can also be determined by dissolving the stearates in diluted sulphuric acid, precipitating the calcium as oxalate and determining the quantity of the latter by titration with N/100 potassium permanganate. But this alternative offers no visible advantage over our method so far as accuracy is concerned and the additional work which it involves is certainly not an asset.

The following solutions are necessary for carrying out the analysis:

1. N/100 ammonium stearate in an excess of stearic acid. Dissolve 5.5 gm. of stearic acid in as little cold absolute alcohol as possible. Gradually pour the solution into a 1000 c.c. measuring flask to which have been previously added 100 c.c. N/10 ammonium hydroxide and 400 c.c. of water. Shake well after every addition, then fill up to the mark and shake well. Most of the free stearic acid will be found after awhile floating on the top of the liquid. The liquid must be shaken every time a portion of it is to be withdrawn. The excess of stearic acid is necessary in order to prevent the hydrolysis of the calcium and magnesium stearates.

2. Saturated stearic acid solution. Dissolve 1 gm. of stearic acid in as little absolute alcohol as possible. Pour slowly into 1000 c.c. of distilled water, shaking well after every addition; then filter off the undissolved stearic acid.

3. N/100 sodium hydroxide.

4. N/100 sulphuric acid.

5. Hydrochloric acid solution of benzidin hydrochloride. Take 9.32 gm. of benzidin hydrochloride (represents 6.7 gm. of the base), add 25 c.c. hydrochloric acid (Sp. Gr. 1.12), transfer the liquid to a 1000 c.c. measuring flask, rinse the beaker with water and fill the flask up to the mark. One c.c. of this solution corresponds to 3.57 mg. of sulphuric acid.

We will now give a detailed description of our method.

Twenty-five c.c. of whole "oxalated" blood (citric acid is to be used) are oxidized in the previously described way, the solution concentrated, transferred to a platinum dish or large crucible which is placed in an inclined position on a triangle with the cover inclined against the upper

edge of the dish and resting on the triangle. The flame is directed against the cover. Fumes of sulphuric acid begin to come off. The flame is then shifted towards the base which is heated to a dull red heat until the white fumes are no longer visible. After the mass in the crucible has been allowed to cool down, a little ammonium carbonate is added which will convert the excess of sulphuric acid into ammonium sulphate which is volatile. The residue is dissolved in as little water as possible, the filtered solution transferred to a beaker of 100 c.c. capacity, the crucible rinsed with several smooth portions of water, care being taken that the total volume of water should not be more than 25 c.c. We now add 25 c.c. of the above described ammonium stearate solution shaking the latter well before addition, filter off the precipitated stearates into a dry weighed Gooch crucible, wash the beaker and precipitate with small portions of the aqueous stearic acid solution described previously, altogether 175 c.c., dry at  $90^{\circ}$  C., wash with about 50 c.c. of petrol ether in small portions, dry at  $120\text{--}130^{\circ}$  C. until weight becomes constant, and weigh.

The stearates are now dissolved on the Gooch crucible in exactly 10 c.c. of N/100 sulphuric acid and the residue washed in about 40 c.c. of distilled water, using 10 c.c. at a time. The clear solution is then titrated with N/100 sodium hydroxide using methyl orange as indicator. The number of c.c. of N/100 sulphuric acid found is deducted from that which was originally added to the stearates and is equivalent to the amount of stearic acid contained in the stearates.

*Calculation.*—In the first place, we have to determine the molecular weight of the stearic acid used as ammonium salt for the precipitation of the earth alkalis. There are several brands of stearic acid on the market, many of them labelled C.P. But, it seems that there is no clear conception in the minds of their manufacturers as to what is a chemically pure stearic acid. The molecular weight of almost all the brands of stearic acid (with the exception of one) is 269-272, indicating that they are probably mixtures of stearic acid with palmitic acid. (Molecular weight of stearic acid is 284.4, that of palmitic acid is 256.3.) The melting point is  $50\text{--}53^{\circ}$  C. (Melting point of stearic acid is  $69.32^{\circ}$  C. and that of palmitic acid is  $63.4^{\circ}$  C.) The only stearic acid which has the correct molecular weight and melting point is the one manufactured by the Eastman Kodak Co., Rochester, N. Y. But it is too expensive and any other acid, labelled C.P. can be used instead if the molecular weight is determined correctly.

This is done in the following way:

Five grams of stearic acid are weighed out accurately on the analytical balance and dissolved in about 50 c.c. of absolute alcohol, which has been previously distilled over calcium oxide in order to render it absolutely neutral. The solution is transferred to a 100 c.c. measuring flask, the beaker is rinsed out several times with small portions of warm absolute alcohol which are transferred to the flask.

The latter is allowed to cool, whereupon it is filled up to the mark with absolute alcohol and shaken very thoroughly. Ten c.c. are titrated with



N/10 sodium hydroxide using phenolphthalein as indicator. After 10 c.c. of N/10 sodium hydroxide have been added and the end point is not yet reached, it is necessary to add about 10 c.c. of alcohol to prevent hydrolysis of the formed stearate.

Let us suppose that 19 c.c. of N/10 sodium hydroxide were used up to neutralize the 10 c.c. of the alcoholic solution of stearic acid. These 10 c.c. contain 0.5 gm. of stearic acid and are equivalent to 19 c.c. of N/10 sodium hydroxide or to 0.076 gm. sodium hydroxide. We have the following formula:

$$\begin{array}{ccccccc} 76 \text{ mg. NaOH} & \text{are} & \text{equivalent} & \text{to} & 500 \text{ mg. Stearic acid} \\ 40 & \text{“} & \text{“} & \text{“} & \text{“} & \text{“} & \text{“} \\ & & & & \text{“} & \text{“} & \text{“} \end{array}$$

$$X = \frac{500 \times 40}{76} = 270.5, \text{ molecular wt.}$$

Let the total weight of the precipitated stearates be A, the number of c.c. of N/100 sulphuric acid used up to decompose the stearates be V, (V is the difference between 10, the number of c.c. of N/10 sulphuric acid added to decompose the stearates, and the number of c.c. of N/100 sodium hydroxide used up to neutralize the excess of the sulphuric acid). V thus represents the number of N/100 stearic acid which were used up to precipitate the stearates.

$$\begin{array}{l} 1 \text{ c.c. N/100 stearic acid} = 2.7 \text{ mgs. S.A.} \\ V \text{ c.c. N/100 stearic acid} = V \times 2.7 \end{array}$$

Let the amount of calcium stearate be X, that of magnesium stearate is then A-X. The molecular weight of calcium stearate is 579, that of magnesium stearate is 563.

$$\begin{array}{rcl} \frac{\text{Stearic acid}}{\text{Calcium Stearate}} & = & \frac{541}{579} \\ \frac{\text{Stearic acid}}{\text{Magnesium stearate}} & = & \frac{541}{563} \\ \frac{541}{579} \times X + \frac{541 (A-X)}{563} & = & 2.7 \times V \\ 541 \times 563 \times X + 541 \times 579 \times A - 541 \times 579 \times X & = & 579 \times 563 \times 2.7 \times V \\ X = \frac{541 \times 579 \times A - 579 \times 563 \times 2.7 \times V}{541 (579 - 563)} & = & 541 (579 - 563) \end{array}$$

Next follows the determination of potassium and sodium. The filtrate from the precipitated stearate is evaporated to complete dryness and the residue heated gently in order to destroy the ammonium stearate. It is then taken up in a little hot water, transferred to a weighed crucible, the water is evaporated on the water-bath and the crucible dried and weighed, the drying and weighing being repeated until there is constant weight. Having thus determined the total amount of the alkali sulphates, we now determine the amount of the sulphuric acid present in both of them.

To the solution of the sulphates are added 25 c.c. of the previously described benzidin hydrochloride solution, stirring vigorously. The benzidin sulphate is precipitated and filtered off after ten minutes, using gentle suction. The last portions of the precipitate are transferred to the filter

with the aid of small portions of the clear filtrate and then the beaker and the precipitate are washed with 20 c.c. of cold water, added in several portions. The precipitate is transferred with the aid of 50 c.c. water to an Erlenmeyer flask and the latter shaken until an homogeneous mass is obtained. The flask is kept closed during shaking, closed with a rubber stopper which is later removed and rinsed off with water. A drop of phenolphthalein is added, the water heated to about 50° C. and titrated with N/20 sodium hydroxide. When the end point is nearly reached, the liquid is boiled for five minutes and the titration is then finished.

*Calculation.*—The calculation is carried on along the same line as that of the earth alkalis.

Given: Total amount of sulphates = A  
 No. of c. c. of N/20 NaOH  
 used in titration = V  
 Amount of  $K_2SO_4$  = X  
 Amount of  $Na_2SO_4$  = A — X

The amount of sulphuric acid present in both sulphates is equal to:

$$\frac{H_2SO_4}{K_2SO_4} \times X + \frac{H_2SO_4}{Na_2SO_4} \times (A - X) =$$

$$0.563 \times X + 0.696 (A - X) = 4.9 \times V \text{ mgs.}$$

$$X = \frac{696 A - 4900 V}{133}$$

We wish to thank Dr. Max Kahn for suggesting this problem, and for his advice during the course of the work.

## A MODIFICATION OF THE FOLIN-WU BLOOD SUGAR METHOD\*

BY SERGIUS MORGULIS, A. C. EDWARDS AND ELIZABETH A. LEGGETT,  
 OMAHA, NEB.

RECENTLY S. R. Benedict published a new method for the determination of uric acid in blood filtrates, employing an arseno-tungstate solution. We found that by a slight modification we could adapt this uric acid reagent for blood sugar determination. By this new reagent we could obtain values identical with those yielded by the Folin-Wu method, but the color developed with our reagent is about 1.7 times as deep and is of a quality of blue more easily read in the colorimeter.

In searching for a reagent which would give more color than the Folin-Wu reagent we were prompted by a desire to find a means of determining sugar in a single drop of blood (0.05 c.c.). Although we were successful in that by this new reagent a strong color is obtained even with such small amounts of blood, the variations in the results—in our experience, at any rate—were so great as to practically nullify the significance of blood sugar determinations

\*From the Department of Biochemistry, University of Nebraska, College of Medicine, Omaha, Neb.  
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made on single drops. We are aware of the fact that good success has been recorded by others who measured the sugar in drops of blood by the Folin-Wu method. In our judgment the extra care which must be exercised in working with minute quantities, and the refinement of a technic suitable for such microchemical work practically preclude the use of these methods in clinical procedure as a general routine. There have been, of course, also so-called "drop methods" described, but the amount required for a determination is so great (about 0.5 c.c.) that we find it much simpler to obtain the blood for analyses from a vein by means of a needle in the usual way.

The blood filtrates are prepared exactly as outlined by Folin-Wu in their excellent system of blood analysis. Depending on the amount of sugar expected, one or two c.c. of the filtrate are boiled with two c.c. of the alkaline copper sulphate for six minutes in Folin's sugar tubes. So far, therefore, the procedure is precisely the same as that of Folin-Wu. If the blood contains more than 0.15 per cent sugar we take only one c.c. of the filtrate.

As soon as the tubes are removed from the boiling water-bath we add to it one c.c. of our modified sugar reagent. This is prepared as follows: 100 grams of sodium tungstate is dissolved in about 600 c.c. of water, then 50 grams of pure arsenic pentoxide is added to the solution. When the material is completely dissolved, 25 c.c. of 85 per cent phosphoric acid and 20 c.c. of concentrated hydrochloric acid are added and the mixture is boiled for twenty minutes. After cooling, it is made up to one liter. This is the uric acid reagent made according to Benedict's directions. The sugar reagent is prepared from this by adding 8 c.c. of concentrated hydrochloric acid for 100 c.c. of the arsenophosphotungstate reagent.

Although the sugar reagent can be prepared satisfactorily with smaller amounts of hydrochloric acid, we prefer to use the stronger acid mixture, as it dissolves more easily and quickly the cuprous oxide resulting from the reduction of  $\text{CuSO}_4$  by the sugar. Increasing the proportion of acid beyond 8 parts for 100 parts of the uric acid reagent is to be avoided as with the stronger acid mixtures weaker colors are obtained.

After the proper amount of the acidified reagent has been added the tubes are cooled by submerging in cold water for three minutes. One c.c. of an exact 10 per cent sodium hydroxide is now added and the contents of the tube diluted with water after standing for five minutes. We generally match the color five minutes after dilution.

The results by our modified procedure agreed with those obtained on the same material by the Folin-Wu method. The comparisons were made with known sugar solutions and with blood filtrates. For a long time all blood sugar determinations in this laboratory were made by both methods simultaneously and invariably with the same result. A few examples will suffice to demonstrate the agreement between the two methods:

Folin-Wu Method

21.4  
17.6  
18.3

Modified Method

21.4  
17.9  
18.4

The uric acid content of the blood does not interfere with the sugar reaction. We applied the modified method both to artificial mixtures and to blood filtrates containing variable amounts of uric acid, but in no instance did we find that the sugar values as determined by either the Folin-Wu or by our method were affected.

A sugar determination was made on a blood filtrate and by both methods was found to contain 109 mg. per 100 c.c. of blood. The filtrates were then diluted with a uric acid solution 1 to 9 and 2 to 8 parts of the filtrate. We thus obtained mixtures corresponding to bloods containing 5 and 10 mg. of uric acid per 100 c.c. blood in excess over the amount present originally in that blood. Sugar determinations were again made by the two methods. In the first mixture we found 98.5 mg. of sugar and in the second 87.7 mg. The calculated amount was 98.1 mg. ( $109 \times 0.9 = 98.1$ ) and 87.2 mg. ( $109 \times 0.8 = 87.2$ ) respectively.

It is obvious, therefore, that by our modified method results are obtained which check with those by the Folin-Wu method and that both are unaffected by the initial amount of uric acid in blood.

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## A PLEA FOR A STANDARDIZED METHOD OF REPORTING WASSERMANN TESTS\*

BY ROBERT A. KILDUFFE, A.M., M.D., PITTSBURGH, PA.

A GREAT deal of effort has been expended upon the various factors influencing the Wassermann reaction and, in the endeavor to enhance the delicacy and specificity of the reaction a multiplicity of technical and manipulative refinements has been introduced.

As a result, practically the only vestige of the original test remaining is the name of its sponsor.

It is natural, under these circumstances, that differing results are at times obtained by different workers using methods which possess a wide potential range of delicacy and specificity and that, particularly in the borderline case where the reaction has often a vital significance, the clinician is sometimes puzzled by the reports made to him.

This fact has led to the recognition of the necessity for the development of a standard method of performing the test which, when generally adopted, may be expected to produce a uniformity of results.

Recent studies along this line give hope that the evolution of a method suitable from the standpoint of delicacy and specificity for standard adop-

\*From the Laboratories of The Pittsburgh Hospital.  
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tion has been achieved. Its general adoption, however, requires time. Confidence in the method must be achieved by a large number of comparative and analytical tests; manipulative dexterity must be acquired, and, above all, personal preferences must be overcome before the method comes into general use.

In the meantime, it would seem advisable to consider the wisdom of an attempt at uniformity in several other phases of the serologic diagnosis of syphilis.

1. Clinicians in general should be at least sufficiently familiar with the test and the factors influencing it to correlate and interpret the findings in conjunction with the other findings of a particular case.

2. The Wassermann test should not be made, as it is in a large proportion of cases, the sole and main avenue of investigation as to the presence or absence of syphilis.

3. The diagnosis of syphilis should not be looked upon as the sole prerogative of either serologist or clinician, but rather as a problem requiring the joint efforts of both, working, not separately, but together.

4. The clinician should insist upon some standard by which to judge the competence and ability of the worker from whom he receives a Wassermann report: he should be able to know if his training has been complete, thorough, and adequate, or if it comprises simply a rule of thumb method of putting a little of "this" in a tube and some of "that" followed by certain other manipulative details. It should be known whether the worker's ability is merely manipulative or whether it includes the ability to interpret as well as to make the test.

5. The clinician should demand something more than a simple "positive" or "negative" report.

In connection with this last, in order that comparisons may be made of various tests: that the relative value and reliability of reports by various methods can be estimated, it seems that a clinician working with a serologist in whose ability he has confidence—and still more so when the serologist is an unknown quantity—is entitled to more information than he often receives.

Different methods vary in reliability and delicacy and, in borderline, weakly-reacting cases, the results are appreciably influenced by variations of technic, and variations in reports on the same case by different workers may be, at times, accounted for by technical differences of method.

For many reasons, therefore, it seems advisable to suggest that a Wassermann report consist of something more than a mere statement of the result arrived at and that some standard, uniform method of submitting reports be agreed upon.

A complete report might rightly include:

- (a) The serum dose, because this factor has been shown to influence the character of the results obtained.

- (b) The antigens used, because various antigens are very decidedly variant in delicacy.

(c) The result obtained with each antigen, separately.

(d) Possibly, until the adoption of a standard method, the character of the incubation: heat, ice-box or ice-water-bath, etc.

(e) The serologist's interpretation in full of the reaction as a whole.

Such a report permits of more accurate interpretation in doubtful cases and has many advantages. If the worker does not happen to use a cholesterinized antigen, for example, his negative reports on cases under treatment are of less value than if this fact is not known. If his serum dosage is too minute, (drops), his reaction is not apt to be sufficiently delicate to detect only traces of reagin. When reports from two laboratories disagree the answer, at times, may be found in a variation of technic, etc., and so on; many other advantages of a complete, detailed report can be thought of.

A justifiable suggestion, also, is that in cases where clinical and laboratory findings disagree or leave an element of doubt, the furnishing of the clinical data to the serologist will not infrequently be of assistance in the final interpretation of the reaction or help to indicate methods through which a diagnosis can be made.

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## BLOOD COUNTS WITH OXALATED BLOOD COMPARED WITH ORDINARY COUNTS\*

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BY ALVIN G. FOORD, M.S., CHICAGO, ILL.

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WHILE doing routine complete blood counts and Wassermann tests on the patients of the Colfax School for the Tuberculous and also handling counts for physicians who had to go considerable distances to obtain blood for counts, the need was seen for a simple way of transporting blood to the laboratory and a way whereby the puncture of the ear might be avoided, inasmuch as the patient is "stuck" once when the Wassermann sample is being taken. Counts directly on the venous blood are not practical for the hemoglobin estimation is not accurate by the Dare or Tallquist methods, and also because the blood clots as a rule before all the necessary procedures can be done. Hence the counts were made to see whether the counting of oxalated blood would be reliable. Miss N. Yarbrough<sup>1</sup> in a report on a limited number of cases claimed that the counts on oxalated blood were practically the same as on blood drawn from the ear or finger, and that the counts were accurate if the blood was kept as long as 3 days. My findings were not as favorable as hers as Table I will show. The method used was to simply eject 1 c.c. of blood from the syringe used in taking the Wassermann sample into a  $\frac{1}{2}$  dram vial containing a little powdered potassium oxalate, shaking thoroughly, after which the blood was taken to the laboratory, shaken again, and then counted (in most cases in from  $\frac{1}{2}$  to 3 hours after drawing) using the same pipette as was used in making the control test from

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\*From the Laboratory of the Colfax School for the Tuberculous, Colfax, Calif.  
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TABLE I

TABLE I												
		EAR BLOOD	FRESH		24 HR. OXAL. BL	FRESH VEN. BL.		POLYS.	L. L. & M.	S. L.	MISC.	COMMENTS
			OXAL. BL.									
Non-Paraffined Tubes	34 cases	13,300	12,380	.....	10,850		Ear Bl. Oxal. Bl.	72.3 71.3	6.3 6.7	19.9 21.0	1.5 1.0	7 out of 26 slides worthless.
	12 cases 5 cases	9,960 11,760	9,960 11,340		9,200		Ear Bl. Oxal. Bl.	71.1 68.9	6.6 6.3	19.4 22.2	2.9 2.6	3 out of 12 slides worthless.
Paraffined Tubes												

the ear. The blood was not kept in the ice box except in the summer. In the spring the vials were kept in a cool spot in the laboratory at about 10 or 15° C.

The control ear blood counts were conducted in the usual way, the pipettes being filled and then closed on the ends with rubber bands. The differential counts were made from smears made on slides, and not by the cover slip method. The blood for these counts was drawn a minute or two before the blood from the vein. Hemoglobin determinations were by the Tallquist scale, and were 10 to 15 per cent lower than the Dare method.

Since the oxalated blood saved for counting only half filled the 2 c.c. vials considerable foam resulted on shaking, and drops of blood adhered to the sides of the vial here and there, which may explain a part of the discrepancies in the table. To keep the blood from adhering to the glass, some of the vials were lined with paraffin. A more even mixing could no doubt be accomplished by nearly filling the vials and having a glass bead in the vial to facilitate the mixing, and also the cells would not be broken up as much. Further experiments will be done along this line.

A few counts were made on venous blood as soon as drawn to compare with the oxalate counts and with the ear blood counts. The results follow:

#### COMMENTS AND CONCLUSIONS

The hemoglobin estimations were in accord in all cases, the red count as a rule slightly lower with the oxalated blood, and the white counts on the oxalated blood were generally a little lower than those from the ear. The difference would hardly be appreciable clinically. It should also be noted that the counts on the fresh venous blood were about the same as on the oxalated blood, and a trifle lower than the ear counts. The oxalate counts are farther off the longer the blood has stood, few being reliable after 24 hours. The reds on the stained smear were poorly stained and were as a rule somewhat broken or disrupted so that study of the morphology was not possible except in a few cells. The chief objection against counting oxalated blood is the finding that so many white cells are broken up, especially the polymorphs, so that the polymorph count is as a rule lower than it should be. Too many slides are absolutely worthless, for the method as conducted to supplant the counting of capillary blood from the ear or finger. The total counts are fairly reliable, but the differential counts are not. However, it seems to me that if the paraffined vials are practically filled with blood so that the air does not make foam which tends to break up the cells, the counts should be quite reliable, especially if the blood is put on ice as soon as possible. However, I doubt whether blood will be satisfactory if it has to be sent through the mails on account of heat it is subjected to in postoffices and mail cars.

The author wishes to acknowledge his indebtedness to Dr. R. A. Peers, whose aid and encouragement made this work possible.

#### REFERENCE

- <sup>1</sup>Nancy Yarbrough: Jour. Lab. and Clin. Med., 1921, vii, 172. Blood Counts with Oxalated Blood.



## LOSS OF SUGAR IN OXALATED BLOOD\*

BY DORA E. BIRCHARD, B.A., LOS ANGELES, CALIF.

IT IS an accepted fact that the sugar content of oxalated blood diminishes on long standing. It is stated, however, that the blood should be kept on ice, and "analyses preferably made on the day of withdrawal."<sup>1</sup> Watson and White<sup>2</sup> have shown that ox blood does not lose materially in sugar in 24 hours, even at room temperature. It was, however, the experience of this laboratory that specimens of oxalated blood in which the protein was not removed within an hour or two after being drawn, showed abnormally low sugar content, even those kept for only 6 or 8 hours on ice. In the case of specimens which had to come from any distance by mail, and under varying temperature conditions, the sugar findings were often so low as to be of no value.

Two possible ways of correcting this difficulty present themselves: If the drop in sugar content is a uniform one with respect to time, a certain factor might be found by which the actual findings could be corrected according to the length of time the blood had stood. On the other hand, some way might be found to inhibit this break-down of blood sugar during the time that must often elapse between the taking of the blood and the actual determination.

Denis<sup>3</sup> suggests the addition of a few drops of commercial formaldehyde to the oxalated blood. This is all right for sugar determinations only, though great care must be exercised to avoid any excess of formaldehyde. In cases where a number of different determinations are to be made on the same filtrate, the presence of formaldehyde tends to interfere with these, particularly the nitrogen determinations. At any rate, if the desired effect can be accomplished without this addition, so much the better. The answer here is the protein-free filtrate, provided the loss of sugar takes place in the whole blood and not in this filtrate.

There are then, these two questions to be answered by the experimental evidence below:

(1) Does the decrease in blood sugar occur as a uniform drop so that a definite "per cent loss per hour" factor can be established, and if so, what is this factor?

(2) Does the protein-free filtrate if made at once, maintain its sugar content at a constant figure, and if so, for how long?

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\*From the Clinical Laboratories of Drs. Grant and Talbott, Los Angeles, California.  
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## METHODS

The Folin-Wu<sup>4</sup> method of blood sugar determination was the one used. A portion of each specimen was deproteinized by the tungstic acid method, immediately after being drawn. The sugar content of the filtrate thus obtained was taken as the standard for that specimen, that is, 100 per cent in

TABLE I  
LOSS IN BLOOD SUGAR IN 24 HOURS IN THE SAME SPECIMEN

SPEC. NO.	MILLIGRAMS OF SUGAR PER 100 C.C. OF BLOOD				PER CENT LOSS		
	AT ONCE	3 HRS.	6 HRS.	24 HRS.	3 HRS.	6 HRS.	24 HRS.
1	157	119	99	97	17.0%	36.9%	38.1%
2	111	78	67	63	*29.9	39.6	43.2
3	129	118	105	85	8.5	18.6	34.1
4	108	94	88	72	12.9	18.5	33.3
5	98	84	80	75	14.2	18.3	23.4
6	125	110	104	93	12.0	16.8	25.6
7	164	138	100	96	15.8	39.6	41.4
8	110	101	91	84	8.1	17.2	23.6
9	103	91	75	68	11.6	27.1	33.9
10	75	67	53	50	10.6	29.3	33.3
Average					12.3%	26.19%	32.99%

\*This figure has been omitted from the 3 hour average because of its very wide variation from the others of its period.

TABLE II  
SHOWING THE VARIATION IN THE LOSS OF BLOOD SUGAR IN 3, 6 AND 24 HOURS

NO.	PER CENT LOSS		
	3 HRS.	6 HRS.	24 HRS.
1	17.0%	39.6%	46.1%
2	16.2	39.6	43.2
3	15.8	36.9	42.2
4	15.7	30.5	41.4
5	14.2	29.3	38.1
6	12.9	28.0	36.0
7	12.4	27.1	34.1
8	12.3	22.0	34.1
9	12.0	18.9	33.9
10	11.6	18.6	33.8
11	11.5	18.5	33.3
12	11.2	18.3	33.3
13	10.9	17.4	26.2
14	10.6	17.2	25.9
15	10.4	17.0	25.6
16	8.5	16.9	25.0
17	8.1	16.8	23.6
18	7.9	14.8	23.4
19	7.4	14.4	22.2
20	5.0	14.2	19.0
Highest	17.0%	39.6%	46.1%
Lowest	5.0%	14.2%	19.0%
Average	11.5%	22.8%	31.9%

calculating the per cent loss. After keeping the oxalated blood on ice for 3, 6, and 24 hours, respectively, a new filtrate was prepared for each interval, and the sugar content recorded and compared with the original finding.

Twenty specimens of human blood were tested after 3 hours, twenty after 6 hours, and twenty after 24 hours. In ten of these, the 3, 6, and 24

hour determinations were made in succession on the same specimens of blood. (Table I.) Most of the specimens had a normal sugar content, only two of this series containing over 130 mg. per 100 c.c. of blood.

Table II represents in a condensed form, all the findings of the entire experiment. The loss percentages of the ten specimens of Table I are here, besides others (cases where it was not possible to do more than one or sometimes two intervals on the same specimen). The three columns are not for parallel specimens therefore, but are simply numbered from 1 to 20 for convenience in reading the table.

TABLE III

SUGAR CONTENTS OF BLOOD FILTRATES MADE AT ONCE FROM FRESHLY DRAWN BLOOD, AND KEPT ON ICE FOR 24 HOURS

SPEC. NO.	MILLIGRAMS OF SUGAR PER 100 C.C. OF BLOOD		PER CENT LOSS IN FILTRATE	PER CENT LOSS IN WHOLE BLOOD (SAME SPECIMEN)
	AT ONCE	24 HRS.	24 HRS.	24 HRS.
1	104	104	0%	25.9%
2	145	146	0	26.2
3	154	148	3.8	42.2
4	158	158	0	34.1
5	97	97	0	39.1
6	110	101	8.1	30.0
7	110	109	0	25.0
8	130	130	0	33.8
9	105	108	0	19.0
10	135	137	0	22.2
	AT ONCE	18 HRS.	18 HRS.	18 HRS.
11	103	104	0	32.0
12	80	80	0	22.5
	AT ONCE	36 HRS.	36 HRS.	36 HRS.
13	460	460	0	21.0

It will be seen from these tables that while it is possible to strike an average, yet this average will be rather widely at variance with the upper and lower limits of its series, and that these upper and lower limits of the three series overlap one another to a marked degree. It is plain therefore, that no absolutely definite factor can be derived, although we may conclude with reason, that the blood does lose about 12 per cent in the first 3 hours, about 22 per cent in the first 6 hours, and about 32 per cent in the first 24 hours. This knowledge may be of considerable value if a sugar determination is being made on blood which has unavoidably stood for some time. If it should be desirable, for the purpose of checking results, to repeat a sugar determination after several hours have elapsed, it must be remembered that the second finding should be approximately 12 per cent lower than the first, if the time between them has been 3 hours, or 22 per cent lower for 6 hours, etc.

For the second part of the experiment, the original filtrate was kept on ice and sugar determinations made on this after 24 hours (in two cases after 18 hours, and in one case after 36 hours). At the same time a new filtrate from the same specimen of blood was made and analyzed, and the

sugar contents of these two filtrates were compared with the original finding and with each other. (Table III.)

In all but two cases there was no decrease in the sugar values in the filtrates made from fresh blood, even though these filtrates were kept for 24 hours, or even 36 hours. A further extension of the time was not carried out. For the two exceptions we have no explanation to offer as yet, other than individual variation; but the risk of loss of sugar in the filtrate is so slight as compared with the certainty of its loss in the whole blood as to be almost negligible.

Herein then, lies the most feasible solution to the problem. The blood should be deproteinized immediately, or within an hour after its removal from the patient. After this treatment it may stand several hours or a day or longer without appreciable loss. If it is not to be kept on ice a few drops of xylene should be added. The removal of the protein requires little time, and only two reagents, the 10 per cent sodium tungstate and  $\frac{2}{3}$  N sulfuric acid. These should be kept on hand in the laboratory which first receives the blood (if it is to be sent elsewhere for analysis), and the protein removed at once. The clear filtrate may then be sent on, or kept until a convenient time, with the above precautions.

#### SUMMARY

1. The sugar content of oxalated blood decreases with a fair degree of regularity, though no absolute factor for this decrease, hour by hour, can be established. Approximate figures, however, may be of considerable value, and these, as determined by the experiments, are as follows:

About 12 per cent for the first 3 hours.

About 22 per cent for the first 6 hours.

About 32 per cent for the first 24 hours.

2. The protein-free filtrate maintains a practically constant sugar percentage, for at least 36 hours, if kept on ice, or with the addition of a few drops of xylene if kept at room temperature.

3. It is important then, that the protein be removed immediately after the blood is drawn. The filtrate may then be kept (preferably on ice) until a convenient time, or may be sent to a distant laboratory for analysis.

I wish to acknowledge with thanks the assistance and valuable advice given by Dr. H. L. White, of this laboratory.

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## EDITORIALS

### *Therapy of Neurosyphilis*

SOON after the elaboration of the Wassermann reaction those parasymphilitic diseases of Fournier which involved the central nervous system, were shown by spinal fluid examination to be syphilitic in origin. This observation was acclaimed, since the possibility now existed that antisyphilitic treatment might induce cure in tabes and general paresis. Soon, however, it was found that the results did not warrant the high expectations and that in spite of intensive intravenous therapy, the majority of cases of outspoken tabes and general paralysis progressed. It was then assumed that the central nervous tissues could not be reached as satisfactorily by intravenous medication as was the case with other tissues, and a method of direct application of salvarsanized serum into the spinal canal was rapidly developed. Even then many cases progressed with little or no improvement. These failures appear due chiefly to the fact that the average case of neural syphilis does not receive intensive treatment until after irreparable parenchymatous damage has occurred. The most successful treatment should be that which is instituted at a time when central nervous involvement can only be recognized by the positive spinal fluid findings.

There is some diversity of opinion as to what therapeutic procedure may best be followed in obvious nervous system infection. In general, three views are held. First, that intravenous medication alone produces satisfactory results; second, that intravenous medication should be combined with spinal drainage; and third, that the administration of salvarsanized serum intraspinally produces the greatest benefit. A few recent articles have called attention to the possibility of increasing neural damage after intravenous medication alone, by a reduction of the concentration of immune bodies. This suggestion has been comprehensively reviewed in an editorial of the January, 1922, number of this *Journal*.

Dereum has been one of the leading protagonists of spinal fluid drainage as against the administration of salvarsanized serum. He points out that the function of the cerebrospinal fluid is preeminently mechanical and that the fluid exists practically alone for the purpose of suspending the brain and cord in a fluid medium and protecting it against trauma. A brain weighing from forty-five to fifty ounces exerts a pressure of but one ounce on its base, due entirely to the supporting action of the spinal fluid. In its chemical constitution, the spinal fluid corresponds more nearly to pure water than does any other body fluid. It contains inorganic salts similar to those of the blood plasma, a trace of coagulable protein and a very small amount of glucose. It is directly continuous through the aqueduct of the cochlea with the fluid of the labyrinth which is generally conceded to exist for mechanical purposes only.

The fluid is distributed through the ventricles and subarachnoid spaces and apparently has no relationship to the perivascular, pericapillary or perineuronal spaces of the cord and brain. It has its source in the choroidal plexuses and possibly also on the serous surfaces of its containing cavities, and leaves through the subarachnoid villi, entering into the venous sinuses. To a lesser extent it also passes out through the lymph sheaths of the cranial and spinal nerves. Dereum states that intraspinal medication was undertaken on the erroneous theory that the nutrition of the nervous system is carried on through the spinal fluid, and that the injection of medicated serum distributes the arsenic into this nutrient medium, thereby circumventing the hypothetical obstruction to the passage of arsphenamine through the choroid plexus.

Craig and Chaney, who also advocate spinal drainage, doubt whether there is much actual obstruction in the choroidal villi to the passage of arsenic. Thus Sicard and Block recovered arsenic from the spinal fluid in three of eleven cases after intravenous injection of arsphenamine. Ravaut obtained similar results in three of thirty-three cases, Hall in two out of eight and again in four out of seven, and Rieger and Solomon in thirty-eight out of one hundred and twenty-three. It must be borne in mind, however, that while arsenic is thus shown to be present in the spinal fluid following intravenous medication, in the majority of cases it was not found. Camp found a questionable trace of arsenic in one of seventeen cases, Benedict in four out of a large series treated, Engman and his collaborators in none of

four cases, and Hall in a third series examined, found arsenic present in one case and absent in nine.

Rieger and Solomon found that in general those patients consistently showing the larger amounts of arsenic in their fluids made the more rapid improvement.

Craig and Chaney agree with Dercum that intraspinal therapy is unscientific because of the rapidity with which chemicals introduced into the subarachnoid space disappear into the general circulation. Thus Rieger and Solomon reported rapid removal of arsenic from the spinal fluid after intravenous arsphenamine, and Hall reports similar rapid disappearance after intraspinal administration. Other substances, such as phenolsulphonephthalein, enter the blood from the subarachnoid space with equal rapidity, appearing in the urine within from four to ten minutes after subdural injection.

The advocates of spinal drainage do not deny the good results obtained by the Swift-Ellis method but maintain that the benefit derived is due not to the substances introduced but to the preliminary spinal drainage incidentally practiced.

The performance of spinal fluid drainage after intravenous treatment was originally based on the assumption that with reduced intraspinal pressure, the arsphenamine circulating in the blood will diffuse more readily into the subarachnoid space. Dercum claims better results than by the Swift-Ellis method, due he thinks, to the more thorough drainage. Every possible drop of fluid is removed each time. Theoretically, the rapid removal of fluid will produce a relative hyperemia of the cord and brain with resulting improved nutrition to the parts. Dercum draws an analogy to the Bier method of hyperemia as used in surgery. If his theory is true, drainage alone irrespective of intravenous treatment might improve the nutrition of the central nervous system with resultant spontaneous improvement.

The presence of arsphenamine in the spinal fluid according to Dercum, is of no significance and probably plays no part in the good results obtained. The difficulty in syphilitic medication lies not in the failure of passage of the spirocheticide through the choroid plexus into the subarachnoid space, but in its failure to pass through the walls of the capillaries situated in the nervous tissue, into the parenchymal cells where the chief damage takes place. The hyperemia resulting from spinal drainage would increase any such tendency to pass through the capillary walls.

Corbus and his collaborators have elaborated a new method in which they believe that they can produce equally efficient spinal drainage without the performance of lumbar puncture. Their work is based on experiments of Foley, who found that after the intravenous injection of hypertonic salt solution, the pressure of the spinal fluid falls markedly. This fall is accompanied by a diminution in brain bulk rather than by an increase. Foley in studying decompression hernia, found that after intravenous injection of 15 per cent saline solution the spinal fluid was almost entirely absorbed into the general circulation as evidenced by recession of the hernial protrusion. This covered a period of two or three hours. The herniation returned within



from six to seven hours after the injection. The increased osmotic tension of the plasma evidently drew fluid rapidly into the blood stream, from all available sources including the subarachnoid space.

Corbus and his associates give 100 c.c. of hypertonic salt solution intravenously followed in six hours by intravenous injection of neoarsphenamine. Treatments are given weekly. They do not draw didactic conclusions from their work and their tabulated results do not allow comparison with other forms of treatment. They apparently assume that penetration of arsenic into the subarachnoid space is an important factor in treatment and claim for their method that this penetration is greater than in any other form of treatment. They found arsenic in the spinal fluid in 93 per cent of their twenty-eight cases.

Rudolf and Bulmer have made chemical tests for arsenic in the various organs and tissues, after intravenous and intraspinal injection of phenarsenamine, and report that after either method practically none can be demonstrated in the central nerve tissues.

Fordyce, who is an ardent advocate of the Swift-Ellis method of treatment, takes vigorous exception to Dercum's conclusions. He points out that Swift and Ellis did not develop their method under the mistaken idea that the nervous system receives its nutrition from the spinal fluid, but rather because intraspinal therapy had been successfully employed in the treatment of cerebrospinal meningitis. Furthermore, they demonstrated clearly that salvarsanized serum possesses definite spirocheticidal properties. Many forms of neurosyphilis have their site in the meninges and are limited to these structures. Meningovaseculitis cannot always be differentiated clinically from paresis and in most cases of true paresis there exists at the same time a meningitis. Positive spinal fluid findings in central nervous system syphilis are due usually to meningeal involvement and this can best be treated by the direct application of a spirocheticide. The more pronounced theluetie meningitis, the more marked will be the benefit derived from intraspinal medication. Fordyce states that numerous cases have been entirely cured and many greatly benefited by Swift-Ellis treatment after the failure of those methods so strongly advocated by Dercum and others. Numerous cases of progressing optic atrophy have been permanently arrested by intraspinal medication after the failure of prolonged intravenous treatment.

Fordyce now omits preliminary drainage and introduces the salvarsanized serum under pressure, with better results than when he was using incidental drainage. Syphilis of the nervous system may exist without coincident clinical or serologic evidence of blood stream infection and in certain of these changes, there is no interchange of the products of syphilis between the nervous system and the blood, nor is there penetration of drugs introduced into the blood stream to the central nervous system. Fordyce states that he has confirmed this statement again and again, and that in these cases remedies applied through the ordinary channels are of no avail.

He emphasizes repeatedly that the problem of the future in neurosyphilis is one of prophylaxis wherein the occurrence of neural invasion



must be recognized before the production of permanent parenchymatous damage.

The prevention of late neurologic accidents in syphilis, however, does not depend solely upon a recognition of early invasion. The theory that inadequate treatment predisposes to late nervous involvement has been discussed in some detail by Keidel. Little is known with regard to immunity in syphilis but we have considerable indirect evidence that the human body does develop some degree of immunity against the *Treponema pallida*. The observation of Colles regarding the apparent immunity of the pregnant mother and that of Profeta, that a "nonsyphilitic" child born of syphilitic parents appears immune, were the first observations based on such a conception. As a rule a syphilitic cannot be superinfected. Pearce and Brown have shown experimentally that insufficient treatment will destroy this immune mechanism and that a second chancre may then be produced even while pathogenic microorganisms persist in the first. They have proposed two "laws," both of which aid in an understanding of late neurosyphilis following inefficient treatment. According to the "law of progression," various tissues of the body are not equally susceptible and reactive to syphilitic infection. Some groups are more susceptible than others, and with a progressing infection there is an orderly sequence from one group to the next. Interference with the course of the infection, as by treatment, may protect some tissues, but unless persisted in may fail to protect tissues higher in the scale. Keidel observes that certain tissues with relatively high resistance to invasion show little defensive reaction after finally becoming involved. If these observations are applied to the central nervous system the latter tissue may be considered relatively insusceptible to invasion with the spirochete, and at the same time unable to satisfactorily develop an immunity reaction when it does become so invaded. Thus the nervous system must depend for its protection upon the presence of immune bodies derived from other tissues. With partial destruction of the treponema, the general immunity reaction becomes less vigorous and the nervous tissues more easily fall prey to the invading parasite.

The second law formulated by Brown and Pearce, "the law of inverse proportions" assumes an inverse quantitative relationship in the intensity of consecutive reactions in syphilitic infection. Thus in the primary sore a defense reaction is set up. If this is vigorous and the chancre is extensive, the later manifestations are likely to be milder if they appear at all. Conversely, with a small, nonreactive chancre, the secondary and tertiary phenomena are usually more extensive. It has long been observed that in secondary syphilis with extensive cutaneous manifestations, central nervous infection is less apt to occur, whereas with but slight cutaneous involvement, later neurosyphilis is more frequent. Treatment which mitigates the severity of the primary and secondary reactions without completely eliminating the infection may predispose to involvement of those more highly resistant tissues such as the central nervous system.

Thus we may use the term accurately when we speak of prophylactic

treatment of neurosyphilis. The efficiency and completeness of treatment of the primary and secondary lesions appears to be of considerable importance in determining later neural invasion. A completely satisfactory standard prophylactic treatment has not been formulated.

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—W. T. V.

### *Aid to Physicians of Ukraine*

IN the January issue we published an editorial briefly describing the situation of the physicians in Ukraine. Believing that many of our readers might desire to aid their colleagues in Ukraine, we shall publish an extract from the letter of Vernon Kellogg, of the National Research Council, in reply to our request for the information contained therein.

"It is a very simple matter to send food relief to any individual or group of individuals in the Ukraine. This can be done by buying food remittances of ten dollars each from the American Relief Administration, 42 Broadway. These remittances are also on sale in many banks. By paying ten dollars you get a certificate in which you put the name and address of the individual whom you wish to benefit and then you send this certificate, keeping a tear-off portion of it as receipt, to your beneficiary in the Ukraine through the regular mails. On receipt of the certificate the beneficiary applies at the nearest American Relief Administration warehouse, or sends his certificate in by mail to this warehouse, and receives the food. There is no difficulty at all about the sending and receipt of these certificates, nor about cashing them in food. The American Relief Administration has up to date sold about eleven million dollars worth of these remittances and over 95 per cent of them have been cashed in Russia. I would suggest that as the American Relief Administration's work in Russia is expected to close early in the summer it would be well to get any food remittances, that you care to send, over promptly."—(Signed) Vernon Kellogg.

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### Erratum

On Page 219 of the January Issue of the Journal, the 18th line should read: " $6 \times 0.17 \text{ G.} = 1 \text{ G.}$ ; Marked depression (part retained)." instead of "died within 12 hours."

## BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, St. Elizabeth's Hospital, Richmond, Va.)

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### *Preventive Medicine\**

THIS is a book by most competent men. While it is written by Canadians and has its direct application to the Dominion of Canada, it is of equal interest and value to physicians in the United States. The preface gives a picture of the contents of the book. After dwelling upon the start in preventive medicine that has already been made in Canada and in this country, the authors state: "Two further steps are necessary. First, the state should provide a service for those to whom the cost of complete health supervision is prohibitive and such a service should be free to all, rich and poor alike, and no stigma of charity should be attached to its acceptance. This service should include provision for antenatal supervision, home visitation, and infant welfare centers to safeguard the health of infants and little children. Medical, nursing and dental supervision in schools and colleges, and finally, clinics for periodic medical examinations of adults. Thus, health supervision would be provided for all those who chose to take advantage thereof: from infancy to old age. The second important point should be: No compulsion! Let those who pay their taxes and help provide the service offered by the state, arrange for their own private supervision should they so desire. Free education is now provided at the public expense. Many there are, however, who pay school taxes but take no advantage thereof. They prefer to have their children educated in schools not provided at the expense of the state."

Following a statement of the problems and aims of preventive medicine, there is discussed in a most authoritative and practical way the recognition and control of the various infectious diseases. Nearly one-half the volume is taken up very profitably with a discussion of water, milk, foods, deficiency diseases, community sanitation, maternal and infant mortality, school hygiene, public health clinics and centers, air and ventilation, industrial hygiene and public health organization. We can commend the book and its teachings to all those interested in preventive medicine. —V. C. V.

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\*An Introduction to the Practice of Preventive Medicine, by J. G. Fitzgerald, M.D., F.R.S.C., Professor of Hygiene and Preventive Medicine and Director Connaught Antitoxin Laboratories, University of Toronto, Assisted by Peter Gillespie, M.Sc., C.E., M.E.I.C., Professor of Applied Mechanics, University of Toronto and H. M. Lancaster, B.A., Sc., Director Division of Laboratories, Provincial Board of Health, Ontario. St. Louis, C. V. Mosby Company, 1922.

# *The Journal of Laboratory and Clinical Medicine*

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## ORIGINAL ARTICLES

### CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

BY A. LEVINSON, M.D., CHICAGO, ILL.

#### INTRODUCTION

THE human body is a biochemical being. Like all living beings, it is not at a standstill; it composes and decomposes, it secretes and excretes, and as soon as it ceases to undergo changes, it is dead. In spite of the constant changes, however, the body retains a certain equilibrium, within wide limits, during health, and a disturbance of the equilibrium outside of the normal limits invariably means disease.

At times a disturbance of bodily equilibrium may be detected easily. At other times, however, both clinical judgment and laboratory technic have to be brought into play in order to establish a diagnosis of the disease.

Of late, a great number of tests have been proposed for various diseases. Some of them are easily applicable, others are not. All tests, however, have to be interpreted correctly; otherwise they are not only of no help, but may even be misleading.

Unfortunately some physicians, recent graduates as well as old practitioners, are not familiar with the interpretation of tests. Laboratory workers are constantly being asked by physicians for "complete blood" or "complete laboratory work," and on furnishing the complete report they are again asked to interpret their results. On the other hand, there is another extreme toward which some physicians, especially young graduates, are tending, namely, to place laboratory tests above all clinical signs and symptoms. The danger of such an attitude becomes evident when a special laboratory test, such as a throat culture, proves negative. We all know how frequently it happens that a culture in a case of diphtheria in spite of all clinical signs pointing to a diphtheria, is negative.



What is true of the interpretation of laboratory work in general is doubly true of laboratory work in pediatrics, for the result of a test that indicates pathology in the adult may be of no significance in the child. Such, for instance, is the case with leucocytosis and lymphocytosis which is a part of the normal blood picture in children.

Laboratory procedures in children present one more difficulty in that it often requires special methods to obtain a specimen from the patient. Such is the case with blood where the jugular vein or longitudinal sinus have to be resorted to in order to get sufficient material for a Wassermann test or for blood chemistry. Such is the case in cerebrospinal fluid where ventricular or cistern punctures have to be employed in order to obtain fluid or to inject serum. The same is even true with urine where special methods have to be resorted to in order to collect a specimen for examination.

In view of the above considerations, it has occurred to me that a course discussing both the value and limitations of laboratory tests in their relation to clinical pediatrics would meet a vital need in the curriculum of the medical school. I was fortunate enough to be able to present such a course to several classes during the last few years at the Northwestern University Medical School.

The course, as I give it, deals with the interpretation of laboratory tests as applied to pediatric practice. It does not aim to teach the student how to perform the tests. It is taken for granted that he has learned the technic during his freshman and sophomore years. The course only teaches the student the interpretation of the most important tests and their application in pediatrics. In studying the blood, the value and limitations of leucocytosis, of relative lymphocytosis, of blood sugar, nonprotein nitrogen, creatinine and urea, of alkali reserve, Wassermann, blood culture and blood grouping are discussed. In studying the urine, the value and limitations of albuminuria, reduction tests, cells, casts, quantitative chlorides, total nitrogen, urea, uric acid, ammonia and kidney function tests, are discussed. Cerebrospinal fluid, milk, stool, and pleural exudate, are studied in the same way. The interpretation of skin tests and of x-ray are also discussed.

A case is presented at every lecture and the tests previously done are interpreted and compared with the clinical findings. Occasionally, difficulty arises as to what value should be attached to certain laboratory results. It is a well-known fact that various workers attach different values to certain tests. Such, for instance, is the case with indican and creatinine in urine, and the fragility of corpuscles in the blood. In such cases I make it a point to tell the students that there is a diversity of opinion as to the value of the test, and that it is on the clinical findings, coupled with the laboratory tests, that a decision should be made.

Although it is my policy not to discuss technic with the students, I find it advisable occasionally to demonstrate or to discuss briefly certain methods of obtaining the specimen. This is usually done in cases that call for special methods on account of the age of the patient, such, for instance, as the method of obtaining blood or urine from infants.

As the course gained popularity it became necessary to give the students some references on the interpretation of the various tests and on methods of obtaining specimens in children. Surprisingly enough, this has become a tremendous task, for the standard text books on pediatrics or those on laboratory methods do not always present the most practical methods of obtaining specimens from children or the clinical application of the tests in children.

It was the necessity of finding a text, and the pressure brought on me by my students and by a number of teachers of pediatrics that called forth the following series of articles which is to be published in the *Journal of Laboratory and Clinical Medicine*. In this outline, I have endeavored to correlate the laboratory tests with clinical pediatrics, with especial emphasis on pediatric interpretation.

The series of articles will consist of the following:

1. Methods of clinical procedures in infants and children. This will include: physical examination, removal of blood from the jugular vein and from the longitudinal sinus; spinal, ventricular and cistern puncture; collection of urine; lavage and gavage; skin tests, etc.

The methods are simplified to such an extent that they can be done by every practitioner of medicine.

2. A description of simple laboratory tests that can be carried out in the ordinary laboratory or even office.

3. Above all, a discussion will be given of the interpretation of various clinical and laboratory tests as applied to infants and children.

The purpose of the various tests, and the clinical application of the results of the tests to infants and children will be discussed. Whenever necessary, emphasis will be laid on the difference between the normal standards in children and those in adults.

The articles are written, not for the pediatrician or trained laboratory worker, but for the medical student and for the general practitioner. An endeavor has been made throughout to be simple rather than complex, practical rather than theoretical.

#### CASE HISTORY

In the case of a very small child the history must naturally be obtained from the parent. In the case of an older child the history should be taken both from the parent and the child. Many a child will describe its symptoms more vividly than its mother, who usually adds some flavor to the story. If possible, the mother should be heard in the absence of the child, so that the child will not be influenced by her story. The history should include the following:

**FAMILY HISTORY.**—This should consist of questions as to the number of children in the family, their ages, and the state of their health; as to the state of the mother's health during the last pregnancy, as to miscarriages preceding or following the birth of the patient; as to the incidence of disease in family with special reference to tuberculosis, syphilis, rheumatism, heart

disease, alcoholism, epilepsy, idiocy and neurosis. All of the above diseases are often familial in character and may have a direct or indirect bearing on the present condition of the patient.

**PERSONAL HISTORY.**—This should include the following questions:

1. *Birth.*—Whether the patient was born at full term or was premature, the latter of which may explain congenital weakness or rickets, whether the birth was natural or instrumental, whether the labor was precipitate or prolonged. Modern research has shown that instrumental or prolonged labor may be responsible for brain hemorrhage in the newly born, which may later manifest itself in a spastic paralysis.

2. *Method of Feeding.*—Whether the child is or was breast-fed and for how long a period. If artificially fed, inquiry should be made as to the kind of food and the frequency of feeding, also as to whether the child was or is getting orange juice or other fruit juices. The method of feeding may be responsible for malnutrition, scurvy, tetany, and intestinal disturbances.

3. *Development.*—Inquiry should be made as to the time the child first sat up, walked, and talked, an important consideration in the diagnosis of rickets, idiocy, and congenital syphilis. Inquiry should also be made as to the time of the eruption of the first tooth and of the subsequent teeth. The latter, however, is not nearly so important in diagnosis as was formerly thought to be; while many rachitic children erupt their teeth late, there are many healthy children in whom the first tooth is not erupted before 12 or 15 months of age. On the other hand, some congenitally weak children erupt their teeth early.

**PREVIOUS DISEASES.**—The history of the following diseases is of the utmost importance:

1. *Intestinal Disturbances*, which may account for malnutrition.

2. *Rickets*, which may be responsible for deformities of the chest or for delayed development.

3. *Tetany*, which may be responsible for convulsions or other neurotic manifestations.

4. *Scarlet Fever*, because of the fact that one attack usually confers immunity, and because of the frequency of nephritis, otitis and adenitis following the disease.

5. *Measles*, which often predisposes the patient to bronchopneumonia and otitis media.

6. *Pertussis*, which is nearly always followed by chronic bronchitis and is often followed by pneumonia and encephalitis. It also predisposes the patient to tuberculosis of the respiratory tract.

7. *Diphtheria*, which may be responsible for myocarditis, stenosis of the larynx and paralysis of various parts of the body.

8. *Tonsillitis and rheumatism*, which may be responsible for chorea and carditis. An effort should be made to inquire as to the presence of the so-called "growing pain," which is often nothing more or less than an acute or subacute articular rheumatism.



9. *Pneumonia*, which makes a second attack more liable, and which is often followed by complications.

10. *Meningitis and encephalitis* which may be responsible for many nervous disturbances, such as convulsions or epilepsy.

11. *Trauma*, which may be responsible for a multitude of affections.

12. *Surgical diseases*, which may cause adhesions or leave other sequela.

It should be kept in mind that there is hardly a disease which does not leave its trace upon the child's body or mind.

**PRESENT HISTORY.**—Careful attention should be paid to the onset of the present disease, whether it was sudden or slow, whether accompanied by fever, chills, convulsions, vomiting, diarrhea, constipation, cough, sneezing or running of the eyes.



Fig. 1.—Method of examination of infant's throat.



Fig. 2.—Method of holding infant for examination of chest.

The mode of onset may at once throw light on the nature of the disease. It may for instance, help decide between measles and scarlet fever. It is well known that scarlet fever sets in suddenly while measles comes on gradually with coryza, conjunctivitis and cough. Vomiting is a cardinal symptom of scarlet fever, although it may be present in other diseases and occasionally is absent in scarlet fever. Convulsions are rather frequent at the onset of infectious diseases in children, and therefore do not necessarily signify an organic disease of the nervous system; fever is present in many diseases, yet the height and type of fever may help in the diagnosis.

#### PHYSICAL EXAMINATION

The patient should be completely undressed, no matter what part of the body is affected.

**POSITION OF PATIENT.**—The position of the child during examination de-



depends a great deal on the age of the child and on the part of the body to be examined.

As a rule, there is no difficulty in examining older children as they can be maneuvered easily to whichever position is desired. The examination



Fig. 3.—General appearance of extremely emaciated infant, one year of age.

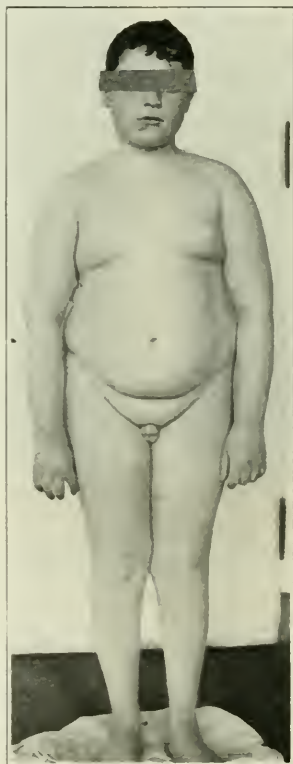


Fig. 4.—Boy of ten presenting Fröhlich's syndrome.

of infants, however, is often beset with difficulties. It is therefore important to select the best position for the purpose of examination. Some physicians prefer to examine infants in the recumbent posture on a table; others prefer to have the infant in recumbent posture on the mother's lap; and still others prefer to have the infant in a sitting position on a table or on the

mother's lap. I believe that the examination of an infant is best accomplished by having the mother or nurse sit on an ordinary kitchen chair and hold the infant on her lap either in the recumbent or in the sitting position, the sitting posture being preferable.

The various parts of the body require different positions on the part of the child. The anterior portion of an infant's chest is best examined by having the mother or nurse hold the baby's legs tight in her lap causing the baby's occiput to lean against her chest and restraining the baby's head with one hand and his arms with her other hand. The same position should be used for the examination of the mouth and throat (Fig. 1).



Fig. 5.—Characteristic posture of patient with decompensated heart.



Fig. 6.—Orthopnea in patient with decompensated heart.

The posterior portion of the infant's chest is best examined by placing the infant's arms around the mother's or nurse's neck (Fig. 2).

To examine an infant's ears, the baby should be placed opposite the light in such a way that one cheek will be leaning on the mother's chest.

**NOURISHMENT.**—The general appearance of the patient will show at a glance whether he is well developed, underdeveloped (Fig. 3) or overdeveloped (Fig. 4). More accurate information can be obtained by weighing and measuring the patient, especially in the case of an infant. In the latter, the weight is one of the best indications as to whether or not the feeding agrees with the patient. In interpreting the weight of the patient, however,

the birth weight, the stature and the racial characteristics should be taken into account. It is natural that what is good weight at three months for a prematurely born infant would be poor weight for a full term baby.

**EXPRESSION.**—A normal child is happy and lively. An idiotic child stares; a child with typhoid is apathetic; with alimentary intoxication is toxic; with meningitis or pneumonia, has a “pinched” expression.

**POSTURE.**—A child suffering from pneumonia usually lies on the affected side; one with peritonitis, lies on his back with his legs drawn up; a patient with cardiac decompensation has to be propped up on pillows (Fig. 5); and in the very advanced stages assumes the orthopneic position (Fig. 6). A patient suffering from asthma has to sit up during an attack. The sleeping posture is also important. Children suffering from adenoids dig



Fig. 7.—Wrinkling of skin in infant suffering from congenital syphilis.

their head into the pillow. Rickety children move from place to place in their sleep and rub their occiput against the pillow.

**SKIN.**—Observation should be made as to the color of the skin, the presence of anemia and jaundice, as to the presence of eczema, urticaria, exanthematous rashes, wrinkling such as is present in congenital syphilis (Fig. 7) scaling, as in overlooked scarlet fever, ichthyosis, and certain local applications, enlarged glands, birthmarks, scars, new growths and edema.

**HEAD.**—The shape of the head, the patency of the cranial sutures, the size and patency of the fontanelles, the presence of craniotabes and parietal bosses, and amount and type of hair and various deformities should be looked for.

The shape of the head may disclose the presence of a microcephalus,

which invariably signifies idiocy; hydrocephalus which is secondary to many organic brain affections (Fig. 8), or square head seen in rickets.

The cranial sutures are open the first week or two of life. After that if open, they signify prematurity, rickets or syphilis.

A normal fontanelle should be on a level with the rest of the skull. A distended fontanelle usually signifies increased intracranial pressure. A depressed fontanelle often signifies extreme weakness. The time of closure of the fontanelles is also important. The posterior fontanelle closes normally between one to three months of age and the anterior fontanelle at sixteen to eighteen months of age. A delay in closure signifies rickets, malnutrition or hydrocephalus.



Fig. 8.—Marked hydrocephalus.

**EYES.**—The expression of the eyes, the width of the palpebral fissures, the length of the eyebrows, the size and equality of the pupils, and their reaction to light and accommodation should be observed. Strabismus, nystagmus, exophthalmus, corneal ulcer, interstitial keratitis, conjunctivitis, ptosis and deformities should be looked for.

The expression of a child's eye will tell at a glance whether the child is active or drowsy, whether he is mentally sound or deficient. An acute observer can often even get an impression of the type of disease by the expression of the eyes.

The width of the palpebral fissure may disclose the presence of a facial paralysis, of an exophthalmus and of mongolian idiocy.

Ptosis is due to paralysis of the third cranial nerve. It is present in



encephalitis, acute anterior poliomyelitis and meningitis. Investigation should be made as to the cause.

Long eyelashes are considered by some observers to signify latent tuberculosis or phthisical habit in children. Racial characteristics must, however, be taken into consideration. No conclusions should therefore be drawn from the mere length of the eyelashes. Markedly dilated or markedly contracted pupils may be due to intracranial pressure. They may, however, be due to the effects of drugs such as the dilation of the pupils by atropine and the contraction of the pupils by opiates.

Unequality of pupils invariably means organic nervous disturbances. The same is true with alteration in the reaction of the pupils to light and accommodation. Strabismus is present in many infants during the first year of life. The condition usually corrects itself. In older children strabismus requires interference either by refraction or operation.

Nystagmus always means organic disturbance.

Exophthalmus is seldom seen below the age of ten, and when present it requires thorough investigation.

Interstitial keratitis should make one suspect congenital syphilis tarda. Acute conjunctivitis is present in measles and in grippe. It may also be due to other infections and to trauma. The etiology of phlyctenular conjunctivitis is still a disputed question. Some claim it to be due to tuberculosis. The presence of phlyctenular conjunctivitis should therefore make one investigate the case further.

Trachoma is rather infrequent in American children but should be looked for. Complete absence of vision is due to a variety of causes, amaurotic family idiocy being one of them.

**EARS.**—The shape of the external ear should be observed as it may give clew to the diagnosis of idiocy. Discharge of middle ear and swelling of mastoid should be looked for. Ear drums should be examined by reflected light for redness and bulging. The latter often solves the diagnosis of an obscure fever.

**NOSE.**—The shape of the nose, deformities and nasal obstructions should be noticed. The shape of the nose often serves as a clew in the diagnosis of congenital syphilis; nasal obstruction is frequent in children suffering from adenoids, sinus infection and general grippe. Discharge from the nose may be due to a mere cold, but may be diphtheritic in character. Every mucopurulent discharge from the nose should therefore be considered suspicious of diphtheria and should be cultured for Klebs-Loeffler bacilli.

In infants, a chronic discharge from the nose should also be suspected of syphilitic snuffles.

**MOUTH.**—The color of the lips should be noted for cyanosis and anemia. for herpes labialis, frequent in pneumonia and meningitis, and for fissures frequent in congenital syphilis.

The gums should be examined for their color, for hemorrhage present in scurvy and in infections of the mouth, and for abscesses.

The presence or absence of teeth should be observed; delayed dentition, decayed teeth and irregular teeth should be noticed.

Delayed dentition is frequent in rickets, although no diagnosis of rickets should be made on mere delayed dentition. Decayed teeth are frequently the source of generalized infection and should therefore be noted carefully. Irregular teeth may be present in rickets and a number of other conditions of malnutrition. Hutchinson's teeth are one of the signs of late congenital syphilis.

The mucous membrane of the mouth should be examined for thrush, stomatitis, and enanthema. It is well known that all exanthema also have an enanthem. Of special diagnostic importance is the presence of Koplik's spots preceding the exanthem of measles.

The tongue should be examined for changes in color. A coated tongue is present in most infectious diseases, strawberry tongue is one of the cardinal symptoms of scarlet fever. The presence of ulcers, cysts, and scars should also be noted; the latter is often present in epileptic patients.

The hard and soft palate should be noted for enanthema, for clefts and other deformities.

The tonsils should be noted for their size, for exudates and abscess. The rest of the pharynx should also be noted for inflammations, exudates and edema. Every suspicious throat necessitates a throat culture, every edematous pharynx should be examined further by palpation with index finger for the presence of a retropharyngeal abscess.

**NECK.**—The neck should be observed for enlargements of cervical glands, present in acute and chronic infections, for the size of the thyroid, for rigidity such as is present in meningitis and meningism, for head-drop which is one of the diagnostic symptoms of an acute anterior poliomyelitis and for anomalies such as thyroglossal cysts.

**CHEST.**—The expansion of the chest and the presence of asymmetry should be noted. Asymmetry is usually present in pleural effusion and cardiac affections. Distended veins over the chest should also be noted as their presence usually indicates an obstruction of the mediastinum by tumor or tuberculous glands, rosary, Harrison's groove occurring in rickets, and retraction of the lower part of the sternum, as occurs in advanced laryngeal diphtheria, should be looked for.

**HEART AND LUNGS.**—Palpation, percussion and auscultation should be carried out the same as in adults. Percussion should be very light. The heart should be outlined and the heart tones noted. It should be remembered that the left heart border in infants is usually outside the nipple line and that the heart tones in infants and in children are louder than in adults. No murmur should be considered "mere functional" unless proved to be so by prolonged observation.

In normal infants the percussion note over the lung is higher pitched than in adults except over the right upper lobe posteriorly. The breath sounds are also louder in pitch than in adults. Any change in percussion and auscultation of the chest should be carefully noted.

**ABDOMEN.**—The shape of the abdomen should be observed for edema, present in decompensated hearts, in inflammations and new growths of liver, and kidneys; for tympanites, present in peritonitis and in many infectious diseases and for retraction, present in miliary and other forms of tuberculosis, also in wasting due to prolonged illness. The abdomen should be palpated for areas of tenderness and rigidity. In infants with severe vomiting reverse peristalsis should be looked for.

Appendicitis in infants and children does not produce the marked rigidity observed in adults. Absence of rigidity, therefore, does not exclude appendicitis. On the other hand pneumonia is frequently accompanied by marked rigidity. Not every rigid abdomen therefore is an operative case.



Fig. 9.—Abdomen in case of decompensated heart.

The liver and spleen should also be palpated for enlargement and tenderness. Enlargement of the liver accompanies decompensated heart, hereditary syphilis and new growths. Enlargement of spleen is frequent in syphilis, leukemia, decompensated heart and acute infectious diseases, especially malaria and typhoid. It is also found in many cases of rickets.

**GENITO-URINARY ORGANS.**—Phimosis and undescended testicles in the male, and vaginitis in the female should be looked for. Hernia should also be looked for.

**SPINE.**—Tender spots and deformities, such as kyphosis, lordosis and scoliosis, should be looked for.

**EXTREMITIES.**—General development, epiphyseal enlargement, such as is

present in rickets; tenderness, present in the early stages of acute poliomyelitis, in grippe, and in scurvy; edema and desquamation should be observed.

**NERVOUS SYSTEM.**—General attitude of patient, type of cry, mentality, choreiform movements, exaggerated or absent reflexes, should be observed.

**OTHER EXAMINATIONS.**—To complete the examination, the temperature should be taken, the urine and blood should be examined, and a tuberculin test should be made. In some cases, a nose and throat culture should be taken. All of these examinations will be discussed under their respective headings.

(*To be continued.*)

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## MIXED TUMORS OF THE UTERUS\*

BY A. J. PETERSEN, M.D., CHICAGO, ILL.

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MIXED tumors of the uterus contain a variety of mesoblastic tissues such as smooth and striated muscle, fibrous connective tissue, fat, bone, cartilage, endothelial tissue and certain undifferentiated tissues derived from the mesoderm. Wilms' monograph in 1900 contains a review of the mixed tumors of the uterus reported in the literature at that time and explains clearly their origin by displacement of embryonal mesoblastic tissue rests along the course of the Wolffian duct. Since Wilms' monograph many other mixed tumors of the uterus have been reported. A summary of fifty of these reports demonstrates that twenty-seven occurred in the fundus of the uterus, the others in the cervix; thirty-two contained cartilage, four bone, fourteen smooth muscle, eighteen striated muscle, five fat, two endothelium, three carcinoma, and almost all sarcoma tissue.

Probably the most characteristic feature of these tumors is their histological structure, that is, their content in a variety of mesoblastic tissues. Almost all of the mixed tumors reported are regarded malignant, although benign tumors are recorded (Perlstein). This malignancy is manifested by a local recurrence after removal rather than by the appearance of remote metastases. The metastases, while infrequent and late, usually do not contain heterogeneous tissues, and occasionally contain tissues not found in the primary tumor. Twenty-eight per cent of the reports of mixed tumors reviewed mention metastases, most of which were confined to the abdomen and pelvis. Tumors of the fundus seem to infiltrate the pelvic tissues later than those of the cervix. Mixed tumors of the uterus have been reported for ages ranging from two years to seventy-five years, although 50 per cent have occurred in women over fifty years of age. Uterine activity seems to have no relationship, for mixed tumors have been found in nulliparous women as often as in multiparous. Clinically they are not easily differ-

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entiated from other malignant tumors of the uterus. Bleeding or leucorrhea is common and pelvic pain is inconstant. Large tumors by pressure may cause symptoms referable to the urinary bladder, or to venous obstruction. Necrotic fragments of the tumor sometimes break away and escape through the vagina. Cachexia, anemia and emaciation appear in the later stages of the disease.

TABLE 1\*

AUTHOR	PLACE OF PUBLICATION	DIAGNOSIS
1. Lobstein	See Meckel, J. F., Handbuch d. path. Anatomie, ii, 311., Leipz., Reclam, 1816.	Lipoma; no microscopic examination
2. Seegar	See Knox, J. H., Bull. Johns Hopkins Hosp. 1901, xii, 318.	Lipoma; no microscopic examination
3. Lebert, H.	Traité d'anat. Pathol. gen. et spec. Atlas., Tom. I, Pl. 16, Fig. 11. Paris, Bailliére et fils, 1857.	Lipoma; no microscopic examination
4. Smith, T.	Tr. Path. Soc., Lond. 1861, xii, 148. See Williamson, H. and Brockman, R. St. L., Proc. Roy. Soc. Med., Lond., Sect. Obst. and Gynec. 1920, xiii, 136.	Fibromyolipoma; microscopic examination in 1920
5. Stroinski, O.	Chicago Med. Review, 1880, ii, 469.	Lipoma
6. Orth, J.	Lehrbuch d. spec. path. Anatomie, ii, 485. Berlin, Hirschwald, 1893.	Lipoma; no microscopic examination
7. Kelly, H. A. S. and Cullen, T. S.	Myomata of Uterus, p. 162, Phila. and Lond., Saunders Co., 1909.	Lipomyoma (2)
9. Brünings	Verhandl. d. deutsch. Gesellsch. f. Gynäk. zu Berlin, 1899, viii, 348.	Lipomyoma
10. Wilms, M.	Die Mischgeschwülste, Heft 2 Leipz., Georgi, 1900.	Lipomatous areas of mixed tumor
11. Merkel, H. 12. Merkel, H.	Ziegler's Beiträge z. path. Anatomie u. z. allg. Pathologie, 1901, xxix, 507.	Lipofibromyoma Lipoma
13. Knox, J. H. M.	Bull. Johns Hopkins Hosp., 1901, xii, 318.	Lipomyoma
14. v. Franqué	Verhandl. d. deutsch. Gesellsch. f. Gynäk. zu Giessen, 1901, ix, 491.	Lipofibromyoma
15. v. Jacobson	Ztschr. f. Heilkunde, xxiii, N. F. 3, Abth. f. path. Anatomie, 1902, p. 139.	Lipofibromyoma
16. Seydel, O.	Ztschr. f. Geburtsh. u. Gynäk. 1903, 1, 274.	Lipofibromyoma
17. Meyer, R.	Ztschr. f. Geburtsh. u. Gynäk. 1903, 1, 274.	Lipoma
18. Pollak, E.	Wien. klin. Wchnschr. 1903, xvi, 68.	Lipofibromyoma
19. Gebhard, C.	Ztschr. f. Geburtsh. u. Gynäk. 1903, xlviii, 111.	Lipomatous areas of mixed tumor

\*This table includes all of the lipomas found recorded after thorough search in the literature.

TABLE I.—Continued.

AUTHOR	PLACE OF PUBLICATION	DIAGNOSIS
20. Spuler, R.	Centralblatt f. allg. Path. u. path. Anat. 1905, xvi, 337.	Lipomatous areas of mixed tumor
21. Ellis, A. G.	Surg., Gynec. and Obst. 1906, iii, 658.	Lipoma
22. Kauffmann, E.	Ztschr. f. Geburtsh. u. Gynäk., Berlin, 1907, lx, 312.	Lipoma
23. Walkhoff, E.	Festschr. f. Rindfleisch, Leipz., 1907, 212.	Lipomyosarcoma
24. Sitzenfrey, A.	Ztschr. f. Geburtsh. u. Gynäk., Stuttg., 1910, lxvii, 32.	Liposarcoma
25. Heddäus, A.	See Perlstein, I., Surg., Gynec. and Obst. 1919, xxviii, 43.	Lipomatous areas of mixed tumor
26. Ley, G.	Jour. Obst. and Gynec., Brit. Emp., Lond. 1914, xxv, 42.	Lipofibromyoma
27. Murray, H. L. & Littler, R. M.	Jour. Obst. and Gynec., Brit. Emp., Lond., 1914, xxv, 26.	Lipomatous areas of mixed tumor
28. Elkin, C. W. W. & Haythorn, S. R.	Surg., Gynec. and Obst. 1917, xxv, 72.	Lipoma
29. Lockyer, C.	Fibroids and Allied Tumors, p. 65, Lond., Macmillan & Co., 1918. See Williamson, H. and Brockman, R., Proc. Roy. Soc. Med., Lond., Sect. Obst. and Gynec., 1920, xiii, 136.	Fibromyolipoma
30. Williamson, H. and Brockman, R. St. L.	Proc. Roy. Soc. Med., Lond., Sect. Obst. and Gynec., 1920, xiii, 136.	Myolipoma
31. Schleussner, R. C.	Proc. N. Y. Path. Soc. 1921, N. S., xxi, 33.	Lipoma

Diagnosis finally depends upon the histological examination of the tumor tissue, although the presence of cartilage or other characteristic tissue in masses large enough to recognize grossly permits a tentative diagnosis, at least. The prognosis after removal, as a rule, is unfavorable and the duration of life after the appearance of symptoms usually is one and one-half to two years. One patient is reported by Peuch and Massabuan (see Perlstein) to have lived six years.

The first of the two mixed tumors of the uterus reported here contains the variety of tissues usually described in such tumors, the second macroscopically contains so much adipose tissue that it seems grossly to be a lipoma, but in the histological preparations there are other tissues than fat cells. Table I contains a list of tumors reported in the literature as lipomas of the uterus, but where careful histological studies have been made there is reference to tissue other than fat cells and the propriety of considering them lipomas rather than mixed tumors is doubtful. However, those tumors containing large amounts of fat seem to be relatively benign, and

have been found accidentally either during an operation or by postmortem examination.

Briefly the salient features of each of the tumors reported here are as follows:

1. Occurred in the body of a uterus surgically removed from an unmarried woman, aged 60 years, with a clinical diagnosis of malignant fibromyoma (service of Dr. T. J. Watkins). The tumor is 8 cms. in diameter, the



Fig. 1.—Illustrating the areas of cartilage and fibrous connective tissue in the mixed tumor of the uterus.

lining of the uterus covering it contains irregular polypi and even macroscopically in the tumor there are large masses of tissue recognized as cartilage. Microscopic preparations contain hyalin cartilage, trabeculae of bone, smooth muscle, alveoli of round and spindle-shaped cells and fibrous tissue. In the sections studied, roughly 2 per cent of the tissue is bone, 35 per cent is hyalin cartilage, 35 per cent is smooth muscle, 30 per cent is white fibrous connective tissue, and 1 per cent is alveoli of round and spindle cells. (Figs. 1 and 2.) The patient died three months after the operation

with recurrence of the tumor in the pelvis and one year after the appearance of symptoms.

2. This tumor was diagnosed clinically a fibromyoma, in an unmarried woman, aged fifty-four years, and the uterus was removed completely (service of Dr. T. J. Watkins). In its submucosa is a light yellow, oily, almost round tumor, 10 cms. in diameter, encapsulated and easily enucleated. The endometrium covering it is edematous, but otherwise it is normal. At the level of the internal os is a fibrous tissue scar of the cervical canal, markedly



Fig. 2.—Illustrating the bone, smooth muscle, and endometrium in the mixed tumor of the uterus.

constricting its lumen. This tumor microscopically contains large masses of fatty areolar tissue separated by bands of fibrous tissue in which there are small groups of cartilage cells and narrow bands of smooth muscle cells. (Fig. 3.) About 1 per cent of the tissue is cartilage and smooth muscle fibers, 5 per cent fibrous tissue, and 94 per cent fatty areolar tissue. The patient is living and well two years after the operation and six years after the appearance of symptoms.

Wilms' explanation of the origin of mixed tumors of the uterus by displacement of embryonal rests along the Wolffian duct is generally accepted. In the female, the Wolffian duct disappears, or only vestigial remnants per-



sist as Gärtner's duct.\* When complete it passes from the epoöphoron through the broad ligament, at first parallel with the fallopian tube, then more diagonally as the base of a triangle whose opposite angle is formed by the uterine portion of the fallopian tube and the body of the uterus.

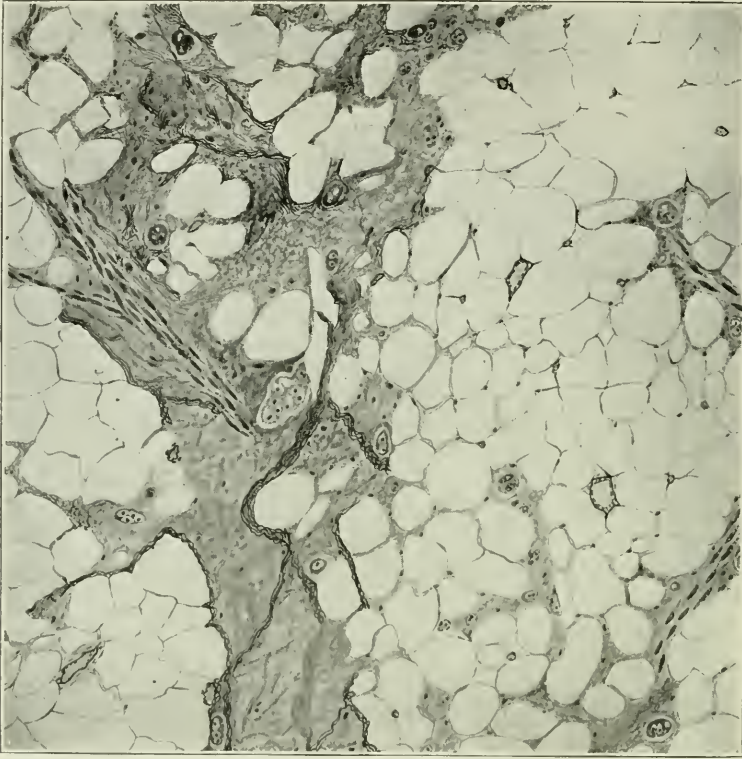


Fig. 3.—Representing the large areas of fat with bands of smooth muscle and fibrous tissue, and areas of cartilage.

Near the level of the internal os the duct passes into the uterine substance, gradually approaching but not reaching the mucosa. Beyond the uterus it may extend in the lateral wall of the vagina to the hymen.

\*Kiebel and Mall: Handbuch der Entwicklungsgeschichte des Menschen. Leipz., ii, 1911.

## THE PSEUDOSPIROCHETES DERIVED FROM RED BLOOD CELLS\*

By EDWIN W. SCHULTZ, M.D., STANFORD UNIVERSITY, CALIFORNIA

INVESTIGATORS have not infrequently been misled by pseudoparasites. A large variety of artifacts, both of endogenous and exogenous origin, have been described as parasites of one sort or another. A pseudoparasite of particular interest, because of its apparently animated behavior, is the spirochete-like body which under certain conditions is extruded from the red blood cell. Its morphology and motility are such that the uninitiated will probably always consider it some new parasite. Indeed, as I shall point out later, several investigators have already given it a place among the parasites and also assigned to it a rôle in disease. It is not unlikely that more such mistakes would be made were the dark-field more generally employed in the examination of blood, or blood fluids. Since much of the literature bearing on this remarkable phenomenon is obscured by misleading titles, a review of the papers which have come to my notice may prove both interesting and instructive. A more general knowledge of the phenomenon among clinical laboratory workers and investigators may serve to forestall further misinterpretation of these spirochete-like bodies.

In 1863, Beale<sup>1</sup> read a paper before the Royal Microscopical Society of London in which he described for the first time this peculiar type of cellular disintegration. The phenomenon in question manifested itself following "the application of a gentle heat" to the preparations. He says: "Under these circumstances long and very fine threads are, as it were, drawn out from the red viscid matter, and these threads exhibited perpetual vibratory movements. I have seen them oscillating in many different places at the same time, like minute vegetable threads developed in the mouth (*Leptomitus*?). Not only so, but many exhibit a distinctly headed appearance; and when these filaments are detached, they certainly very closely resemble bacteria." These changes were observed in both human and frog blood. The following year Preyer<sup>2</sup> described similar observations on extravasated blood of the frog and salamander. He also observed that the phenomenon is promptly induced when a drop of blood is placed on a slide coated with a thin layer of crystallized urea. Schultze<sup>3</sup> in 1865 made a careful study of the influence of temperature on red blood cells, employing a specially designed thermo-objective which was lowered into a small moist chamber containing the preparation; essentially a warm stage of the present day. By this means he was able to observe accurately the effects of given increases of temperature on the cells. His studies were limited to human blood. The most pronounced effects were noted when the temperature reached 52° C. Marked

\*From the Department of Bacteriology and Experimental Pathology, Stanford University.  
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alterations in the shape of the red cells occurred. In some cases the corpuscles became drawn out into long cylinders, with terminal enlargements; other corpuscles were transformed into long threads with bead-like variicosities, and still others developed one or more long filaments. These filaments showed lively serpentine motion, even after they became detached from the corpuscle. Schultze also observed that the red cells in the blood from patients with high fevers were much more prone to undergo deformities than those in blood from normal individuals. This was also observed subsequently by other investigators. Arndt<sup>4</sup> in particular noted the greater facility with which the red cells from febrile cases gave rise to filamentous extrusions, and other types of disintegration. He was especially impressed by the marked deformities of the red cells from a case of relapsing fever, which he observed at the time of a paroxysm. Because of his observations he doubted the parasitic nature of the spirochetes just previously described in relapsing fever, considering it more likely that they were produced by the red cells under the influence of fever and consequent nutritional disturbances. Such views were also expressed by Guttman<sup>5</sup> the following year. Albrecht,<sup>6</sup> on the other hand, with the aid of these disintegration products pieced together a well thought out life cycle for *S. obermeieri*.

The phenomenon has also been described a number of times in more recent years, and in practically every case as observations which were new to the investigator. One of the most interesting of these is by Nuttall and Graham-Smith<sup>7</sup> in 1907. Their observations were made while studying canine piroplasmiasis. It is worthy of note that the phenomenon was the cause of much trouble to these experienced investigators, until they "succeeded in demonstrating that these bodies are not parasites, but degeneration products of red blood corpuscles." The cause was traced to retained heat in the platinum loop used in transferring a drop of blood to a slide. The effect of heat on red blood cells was thereupon carefully studied. They observed that in human blood the most marked changes occurred when the temperature was raised to between 51.5° and 52.5° C. The corpuscles of guinea pigs, rabbits and dogs withstood a slightly higher temperature (54° C.). Later in the same year, Eve<sup>8</sup> described the origin of these spirochete-like bodies from the red and white cells in several pathologic cerebrospinal fluids. The first instance was in the cerebrospinal fluid of a chimpanzee affected with cerebral syphilis. "Attached to some of the red corpuscles were minute filaments showing active streaming movements, and resembling in some ways the living *Spirocheta pallida*." Motile filaments were also observed in the spinal fluid of one out of ten general paralytics he examined. Most of the remainder showed other degeneration products—globular bodies united by short filaments, etc. He observed that white as well as red cells may flagellate. This has also been pointed out by other investigators (see Balfour<sup>10</sup>), but flagellation of the red cells seems to be far more common. Porter,<sup>9</sup> also in 1907, observed the phenomenon in blood obtained by finger puncture, which he sealed between a slide and cover-slip and left for a number of hours at room temperature. In addition to corpuscles "with fine hair-like processes,"



he observed bodies which were "not unlike a tadpole in appearance, and moved by a lashing of the tail," and likewise other odd forms, much like those described by earlier workers. Balfour<sup>10</sup> in 1911 described the phenomenon in an excellent article on blood artifacts, which every novice should read. He refers to the various types of corpuscular disintegrations as the "chain, dumb-bell, droplet, and filament phenomenon," a term which is indeed descriptive. Balfour points out that while the influence of heat on the cells is an important factor in the production of these filaments, "it is evident that they may be seen by the dark-field method when there is no possibility of the blood temperature having been raised, at least to any appreciable extent." Balfour's references to the observations of Crawley, Ross and Macalister, and Buchanan, I have been unable to locate in the literature. Seidlin<sup>11</sup> the following year in a report on his yellow-fever studies states that he observed by the dark-field method "some peculiar filaments" in the blood of several cases of yellow-fever, and later in the blood of normal individuals. "They appeared at first as small, extracorpuseular rods, which in some cases seemed to take their origin from small, free rings; they developed subsequently into fine filaments, the length of which varied from one-fourth to almost the entire diameter of an erythrocyte. They had generally, a distinct dot in one or, more frequently, in both extremities, and were very motile, possessing undulating, distinctly progressive movements." He did, however, not recognize the ultimate source of these bodies, as the following lines reveal: "The nature of these filaments remains entirely obscure. They have certainly nothing to do with the red blood corpuseles, and are thus different from the bodies described by Nuttall and Graham-Smith (1906), and Balfour (1911). The only relation to blood elements which I can admit as possible, is that they might take their origin from platelets; the ring-shaped bodies, from which they were seen to develop were not wholly unlike these elements. This origin, however, would not account for their peculiar shape and characteristic movements, features which surely suggest living organisms." What he undoubtedly observed were the flagellated droplets extruded from the red blood corpuseles. These are common in blood subjected to momentary heat. That they "suggest living organisms" is certainly true. Gastou,<sup>12</sup> in his little book on the dark-field, published in 1912, illustrates the common morphologic types of these filaments. In 1920, Eberson,<sup>13</sup> without reference to any of the earlier work, again described the phenomenon. He produced these filamentous extrusions from the red corpuseles by altering their physicochemical environment; that is, by changing the tonicity or hydrogen-ion concentration. He found, in fact, that simply transferring the cells from their normal environment was all that was necessary to induce the phenomenon. He discovered, however, that it was distinctly accelerated by certain tissue extracts. A little testicular extract caused an abrupt transformation of the cells. He mentions that "extremely tenuous, filamentous forms" are occasionally seen in testicular fluid obtained by puncture; "findings that have led some workers to suggest



these appearances as phases of the life-cycle of the organism causing syphilis." This again illustrates the practical bearing of the phenomenon.

To illustrate how easily the unwary investigator may be misled by these bodies, let me cite some misinterpretations which have been made. In 1892, Thoinot and Calmette<sup>14</sup> announced that they had discovered actively motile, flexible, threads in the blood of typhus fever patients and cadavers. These bodies measured from ten to thirty microns in length and moved in a rapid serpentine fashion among the corpuscles. These filaments often showed more or less prominent terminal enlargements. The longer filaments, in addition to the terminal enlargements, showed secondary enlargements scattered along intermediate points. These, they state, were of the same refractile character as the terminal enlargements, but of a smaller size. These I believe were undoubtedly the varicose forms frequently present among degenerating red blood cells. Similar filaments were seen attached to red blood cells, but these showed no active motion. What they undoubtedly observed here was the production of the filaments. Unfortunately their illustrations were sketched from memory some time after the observations were made, and do not depict the filaments in their true form. Their description, however, leaves no doubt in my mind as to what they actually saw. It is significant that though they were fully cognizant of the aforementioned observations by Schultze, Preyer, and Arndt, they were nevertheless inclined to regard these bodies as "éléments spécifiques." Two months later a paper by Lewaschew<sup>15</sup> appeared showing that this investigator had fallen into the same error. Lewaschew even went so far as to propose the name "Spirochete exanthematicum" for these filaments. He speaks of cocci, cocci with threads, and free threads, considering it probable that these are stages of the same organism. Some of the threads showed varicosities; others a single terminal enlargement, while the free threads were of uniform contour. It is interesting that he observed these bodies in increasing numbers as the disease progressed, and that the number and their activity rapidly decreased as the temperature dropped, and after the crisis almost entirely disappeared. I have already referred to the apparent relationship between fevers and this phenomenon. In 1919 I examined about fifty specimens of blood from cases of typhus fever with the dark-field. These pseudospirochetes were present in large numbers in practically every specimen examined.

In 1912, Dr. Chambers<sup>16</sup> described them as a new spirochete of the human blood stream. These she observed in the blood from cases of Graves' disease, and in the blood from healthy individuals. She described them as actively motile; quite variable in both length and thickness; body soft and flexible, forming open curves, which were constantly obliterated and reformed, and possessing terminal enlargements. She believed that the thinner forms were difficult to distinguish from *Treponema pallidum*. Some were seen to be attached to red blood cells. She believed that she had succeeded in cultivating them. The "spirocheta," however, multiplied only in the original blood clot. This to my mind means that as the corpuscles degenerated, pseudospirochetes were formed. I have observed them in large numbers

in the blood from typhus fever patients which had been left in an incubator overnight or longer. Dr. Chambers stained the "spirocheta" by the Leishman, Giemsa, and Levaditi's pyridin methods. Porter<sup>17</sup> shortly after pointed out the true nature of the supposedly new organism Dr. Chambers described. He says: "The bodies Dr. Chambers described I observed originally in the urine and I thought I had discovered a new organism till I found them in the blood and discovered what I believe to be their true significance. In certain anemias where poikilocytosis is well marked, the pointed end is an extrusion from the corpuscle and has the power of movement. This elongates and is finally detached into one of Dr. Chambers' thick form of 'spirocheta' and by careful observation this will be found in time to elongate into finer 'spirocheta.' "

In 1916 Korbseh<sup>18</sup> in the German Army described what he presumed was

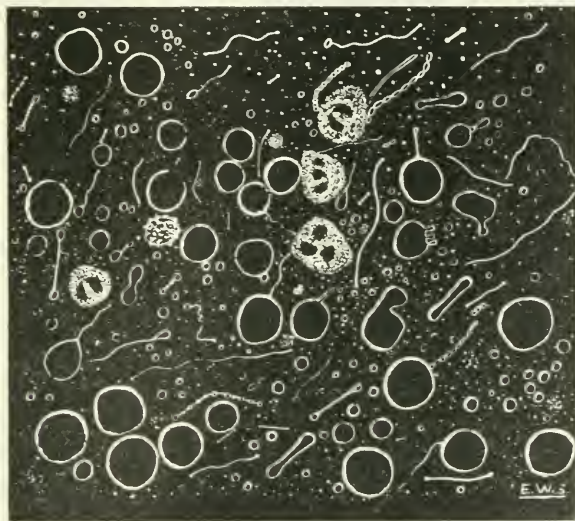


Fig. 1.—Pseudospirochetes and other products of corpuscular disintegration following the application of a hot platinum loop to a drop of blood.

a spirochete in the blood of a then new disease, resembling relapsing fever, later designated Wolhynian fever, trench fever, etc. Because of the efficacy of arsenic therapy in the disease he sought for a spirillum as the most likely cause. His search for spirillum was rewarded. He observed actively lashing filaments, measuring about ten microns in length, among the erythrocytes. In the Giemsa preparations these stained a pale grayish-blue, occasionally a distinct reddish violet, and showed for the most part reddish granules. There were both stretched and coiled filaments. The bodies were found on the febrile days, especially towards evening when fevers are generally highest. We see again the relationship of the phenomenon to fevers, though I have no doubt that diligent search with the dark-field will reveal them at any time. That the phenomenon is prominent also in trench fever is indicated by a remark made by Jungmann and Kuczynski<sup>19</sup> in a report of their studies

on the etiology of the disease, to the effect that they observed the production of the pseudospirochetes in abundance.

Although one unfamiliar with these pseudospirochetes might take them for new parasites, there should be no difficulty in differentiating them from *Treponema pallidum*. Shortly after Eberson's paper appeared, Sutton,<sup>20</sup> as a safeguard, advised the use of distilled water, instead of physiologic salt solution, which I<sup>21</sup> had recommended, as a vehicle in aspirating the pulp from regional lymph glands for dark-field examination in suspected primary syphilis. However, laking the blood does not entirely prevent the production of the pseudospirochetes. I have observed them in blood laked purposely to determine this point. Therefore, the real safeguard rests on an acquaintance with these bodies. Fortunately they are rarely seen in the routine dark-field examination for primary syphilis. In the course of more than fifteen hundred dark-field examinations for primary syphilis made while in the army, I observed these spirochete-like bodies only twice. I never observed them in the aspirated pulp from regional lymph glands. The reason for the rarity of pseudospirochetes in the usual dark-field examination for syphilis undoubtedly rests on the brevity of the procedure. Though red cells may flagellate immediately after they are subjected to deleterious influences, under more tolerable conditions this takes place slowly.

These pseudospirochetes vary considerably in morphology. They vary in length from less than five microns to more than forty microns. Usually they measure between ten and twenty microns in length. No relationship exists between length and thickness. Some are very long and thin, while others are short and thick. Between these two extremes, may be found all possible combinations of length and thickness. The majority measure about 0.5 micron in thickness, and when viewed with the dark-field, the entire thickness of the body is illuminated. The exceptionally thick ones show only marginal illumination, as do red blood cells, droplets, and the larger bacteria. The filaments usually have more or less marked terminal enlargements; this is in the form of either a dot, bulb, or globule. In some these are present at both ends, in others at one end only, and rarely the filaments terminate bluntly without any enlargement whatever. The filaments, however, never taper to a point. The terminal dots and knobs are slightly more refractile than the rest of the filament; the globular ones, however, show only marginal illumination. The filaments are otherwise of uniform thickness and even contour. They are never cork-screw spiral in shape. The filaments eventually develop varicosities and break up into coccoid bodies. These varicose forms often resemble chains of streptococci. Besides the free filaments, one may observe various bizarre bodies, globoid bodies with flagellae, chains, dumb-bells, etc.

The movements of these pseudospirochetes give one the impression of living organisms. These vary somewhat, depending upon the dimensions of the filaments. The long delicate threads exhibit a streaming motion, with gentle open curves. Those somewhat thicker exhibit serpentine, or lashing movements. Others, apparently more rigid, make only slight bending move-



ments; while still others lie relatively quiet. The most common movements are serpentine and moderately progressive. These movements are, of course, dependent upon physical laws and not upon living forces.

The differences between these pseudospirochetes and *Treponema pallidum* are briefly as follows: (1) The pseudospirochetes are not cork-screw spiral in shape. (2) They do not have tapering ends. (3) Their bodies are flexible and when in motion form open curves. (4) They never exhibit rotary motion around their long axes, with forward and then backward movements.

A rapid way to demonstrate the phenomenon is to place a small drop of blood on a slide; touch the drop with a hot platinum loop, drop on a cover-slip and examine with the dark-field. To prepare stained preparations, place the drop at one end of the slide; touch with hot platinum loop; smear with the edge of another slide, and stain according to either Jenner's, Wright's, or Giemsa's method. The filaments take the same color as the red blood corpuscles, and correspond in morphology to those observed in the dark-field.

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# THE PHARMACOLOGY OF ISOPROPYL ALCOHOL\*

## A SYNOPSIS OF AVAILABLE DATA

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**I**SOPROPYL, or secondary propyl, alcohol has recently come into commerce, both in America and abroad. Recent articles in technical journals indicate that it is coming into use in Europe as an ingredient of various preparations for cosmetic and external medicinal uses, and give reason to suppose that it will find such use in this country<sup>1</sup>. The question of the physiologic effects of this alcohol, when ingested or applied to the skin or mucous membranes, is therefore timely and of some importance. A thorough search of the available literature, together with the results of some unpublished investigations, is epitomized below.

Isopropyl alcohol is a homolog of ethyl alcohol, having the empirical formula  $C_3H_7OH$ , and isomeric with normal propyl alcohol. It is produced in this country as a by-product of the petroleum or natural gas industry and in Germany by the reduction of acetone.

Isopropyl alcohol is similar in most of its properties to ethyl alcohol. The commercial alcohol, which is a constant-boiling mixture containing 91 per cent alcohol and 9 per cent water, boils at  $80.4^{\circ}$  C. and has a specific gravity of 0.816 at  $20^{\circ}$  C. The odor of isopropyl alcohol is different from that of ethyl alcohol, lacking the vinous quality, while its taste is distinctly disagreeable except in high dilution.

### EARLY INVESTIGATIONS

The value of many of the earlier data on the toxicity of this substance is impaired by the doubtful purity of the alcohol employed.

Dujardin-Beaumetz and Audigé<sup>2</sup>, by subcutaneous injection in dogs, found both isopropyl and normal propyl alcohols to be about twice as toxic as ethyl alcohol.

Ringer and Sainsbury<sup>3</sup> investigated the depressant effect of various alcohols on the isolated hearts of frogs, and found isopropyl alcohol to be about two and one-half times as toxic as ethyl alcohol.

Efron<sup>4</sup> recorded the reaction to galvanic stimulation of frog's nerve-muscle preparations immersed in the dilute alcohols. He found the toxicity of isopropyl alcohol to lie between that of ethyl and that of normal propyl alcohol.

Schneegans and von Mering<sup>5</sup> administered 4 grams of isopropyl alcohol to a rabbit and observed narcotic symptoms lasting 2 hours, followed by complete recovery.

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Vandeveld<sup>6</sup> compared various alcohols by their effects on plasmolysis in onion cells and on hemolysis in human and bovine blood corpuscles. He found isopropyl alcohol to be from one-third to one-half more toxic than ethyl alcohol, and about two-thirds as toxic as normal propyl alcohol, when used alone. In solution in ethyl alcohol, however, the toxicity of all the higher alcohols was increased.

Kemp,<sup>7</sup> by galvanic stimulation of frog's muscles, showed isopropyl alcohol to be less toxic than normal propyl alcohol.

#### RECENT AMERICAN WORK

Since isopropyl alcohol has come into production on a commercial scale, its pharmacologic properties have been more rigorously investigated.

The work<sup>8</sup> of Dr. David I. Macht of Johns Hopkins University may be summarized as follows:

Comparison of the lethal doses indicates that isopropyl alcohol is twice as toxic as ethyl alcohol when administered intravenously to cats. The comparative depressant action of the alcohols on the isolated frog's heart and on isolated plain muscle (pig's ureter) showed a similar ratio of toxicities.

Rats exposed to air saturated with methyl alcohol vapor died in a day or two, while both ethyl and isopropyl alcohol left them uninjured after several days\*, causing, moreover, no blindness nor defect in vision.

Subsequent work by Dr. Macht<sup>9</sup> shows the minimum lethal dose of orally administered isopropyl alcohol, for cats, to be about 6 c.c. per kilo, while the same dose administered to dogs caused only a narcotic effect. Repeated sponging of the shaved skin of rats, dogs and rabbits with the alcohol caused no toxic symptoms, either local or systemic, nor any interference with the growth of hair. Of a number of rats having the posterior half of the body dipped repeatedly into isopropyl alcohol, practically all survived, notwithstanding possible absorption through the anus.

The narcotic effect of inhaled and of intraperitoneally injected isopropyl alcohol was tested by the behavior of trained white rats in the circular maze. The narcotic dose was found to be exactly half that of ethyl alcohol.

Dr. R. Burton-Opitz, of Columbia University, has also investigated† the toxicity of isopropyl alcohol.

Isopropyl and ethyl alcohols of various concentrations were injected into the jugular vein of dogs, and the carotid arterial pressure recorded. The depressant action of the two alcohols was found to be approximately equal.

Isopropyl and ethyl alcohols were found to have about the same effect when instilled, in various concentrations, into the conjunctival sac.

Rabbits and dogs inhaling air saturated with isopropyl alcohol vapor for an hour showed only a transient unsteadiness of gait.

Administration of isopropyl alcohol by stomach tube to a dog in a dose of 7.7 c.c. per kilo body weight produced severe narcotic symptoms, with complete recovery.

\*Personally communicated.

†Unpublished in extenso, but abstracted in *American Perfumer*, 1921, xvi, 334.

Dilute isopropyl alcohol was found to possess healing properties similar to those of ethyl alcohol when applied to wounds on dogs.

Dr. Burton-Opitz has performed further work on this subject, and will publish it in the near future.

Dr. W. Gilman Thompson\* administered isopropyl alcohol to human subjects. Each of six volunteers drank 25 c.c. of isopropyl alcohol (22.8 c.c. on an absolute basis), suitably diluted. One-half hour after the ingestion of the alcohol a diminution of the systolic blood pressure varying from 6 to 20 mm. was noted. In four cases, there was an increase in the pulse rate of from 6 to 14 beats per minute. Three of the subjects noted a slight dizziness. In no case was there any mental exhilaration such as would be produced by taking ethyl alcohol, nor was there any mental disturbance or muscular incoordination. After two or three hours, all of the men complained of headache, mild and transient in two cases, more severe in two cases, and very severe in the last two so that the subjects did not recover for about 24 hours. Practically all the men felt mentally depressed.

The effect of isopropyl alcohol on the human skin was tested under the supervision of Dr. J. M. Sinclair,† of this Company. Five volunteers wore bandages saturated with undiluted isopropyl alcohol on one forearm, covering an average area of 60 square inches, for four daily periods, aggregating 21 hours for each man. No harmful effects, either local or systemic, were noted, other than a slight maceration of the outermost layer of the epidermis, unaccompanied by irritation.

Isopropyl alcohol has been employed by members of this laboratory, during the past three years, in lotions, liniments, liquid soap, mouth washes and gargles. It has been used undiluted to cleanse small wounds and to dry and harden the skin of hands swollen by hot water or alkali. The present writer has frequently taken sponge baths, from head to foot, in isopropyl alcohol of about 30-50 per cent concentration, which was found to be remarkably cleansing. In no case has any injurious effect been noted. The alcohol has been recently used by several nurses and hospitals for alcohol rubs on a number of patients, under medical supervision. No untoward effects have been reported.

It should be noted that all the recent American work described above has been done with the commercial 91 per cent isopropyl alcohol. All the dilutions mentioned are on this basis, and do not refer to absolute alcohol.

#### GERMAN INVESTIGATIONS

The following recent papers from German medical and technical journals are of interest.

Dr. H. Boruttau of Berlin University<sup>10</sup> bound the shaved abdomens of rabbits and the forearms of human subjects with bandages saturated with 60 per cent isopropyl alcohol. These were worn several hours daily for 14 days. Aside from a slight erythema and superficial maceration, exactly similar in degree to that caused by ethyl alcohol in control experiments, no pathologic symptom or other disturbance of normal health was noted.

\*Personally communicated.

†Personally communicated.

The minimum lethal dose of isopropyl alcohol, by stomach tube to rabbits, was 5 grams per kilo, the same as that of ethyl alcohol.

Boruttau drank 5 c.c. of isopropyl alcohol himself three times, without injury. He also employed it in hand-disinfection and in the preparation of a mouth-wash, without ill effect. He concludes that "there is no reason why isopropyl alcohol should not be employed for cosmetic and hygienic purposes, so long as it comes in contact with the epidermis, or temporarily with the mucous membranes, and no harm need be feared from the accidental swallowing of moderate quantities of this alcohol."

Dr. K. Loeffl<sup>11</sup>, together with two companions, drank a liqueur containing 4 c.c. each of isopropyl and ethyl alcohols. On successive days the dose was doubled, and finally 40 c.c. of a liqueur containing 40 per cent isopropyl alcohol was taken daily for three days. This corresponds to 16 c.c. of pure isopropyl alcohol. No harmful effect was observed. Loeffl says: "I am convinced that no harmful effect can result from the use of isopropyl alcohol for external washes or from the small quantities which may be absorbed through the skin or in the stomach during the use of mouth washes, dental solutions or hair preparations. The use of isopropyl alcohol for these purposes is therefore justified."

Dr. J. Pohl<sup>12</sup> of Breslau University investigated the metabolic transformation of isopropyl alcohol in rabbits and dogs, by analysis of the exhaled air. About 12 to 13 per cent of the original dose was exhaled as acetone and unchanged isopropyl alcohol. Analysis of the liver of one dog killed after twelve hours indicated only a slight retention of the alcohol. Pohl considered that a negative copper-reduction test on the urine indicated the absence of conjugated glycuronic acids. This is in contradiction to the work of Neubauer<sup>13</sup>, who found part of the isopropyl alcohol ingested by rabbits and dogs to be excreted in combination with glycuronic acid.

Pohl states: "I consider myself justified in concluding, subject to the reservation that the exhalation should perhaps have continued longer than 12 hours, that small doses of isopropyl alcohol, incapable of producing narcotic symptoms, are transformed into heat in the body, that is, oxidized, to the extent of over 80 per cent. Isopropyl alcohol therefore approximates ethyl alcohol, of which, as is well known, over 90 per cent is burnt up in the metabolism."

Pohl also performed an experiment on the effect of repeated small doses of isopropyl alcohol on growth. Two puppies from the same litter were given repeated small doses, totalling 224 c.c., of isopropyl alcohol and water, respectively. The alcoholized puppy gained 220 grams more than the other, during two months.

A large dog in nitrogen equilibrium was given 20 c.c. of isopropyl alcohol, and the total nitrogen in the urine determined for a two-day period before and after ingestion of the alcohol. Only an insignificant increase was observed.

The antiseptic and bactericidal action of isopropyl alcohol has been investigated by Bernhardt<sup>14</sup> and others, whose work will shortly be reported by the present writer<sup>15</sup>.



## SUMMARY

The toxicity of isopropyl alcohol is not less than that of ethyl alcohol and not more than twice that of ethyl alcohol. These limits include all the reliable data at hand, by six different observers, using a total of nine different methods, on one or another of six different animals.

The effect of isopropyl alcohol upon the human skin is similar to that of ethyl alcohol.

Different observers do not agree upon the effect of large doses of isopropyl alcohol orally ingested by human subjects. Loeffl's investigators took 16 c.c. daily for three days without discomfort, while Thompson's subjects suffered marked, though not dangerous, depression from a single dose of 22.8 c.c. All are agreed, however, upon the absence of any exhilarating effect similar to that of ethyl alcohol, and upon the somewhat unpleasant taste of isopropyl alcohol. It may, therefore, be safely said that isopropyl alcohol is not potable.

In view of all the foregoing data, no reasonable objection can be made to the use of isopropyl alcohol as a lotion or vehicle for external medication, nor as a constituent of compounds for oral and nasal medication, such as practically all mouth washes, gargles, dentifrices, etc., which do not involve the probability of swallowing more than a few cubic centimeters, at most, of the alcohol.

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## THE PATHOGENICITY OF STREPTOCOCCI FROM DIPHTHERIA THROATS FOR MICE\*

BY LOUIS BRODY AND LLOYD ARNOLD, M.D., CHICAGO, ILL.

SOME interesting changes in the streptococci flora of the upper respiratory tract during attacks of diphtheria are recorded in another paper in this number.<sup>1</sup> One of the most striking differences observed was the change in the limiting hydrogen-ion concentration of the strains isolated from the throats of diphtheria patients. The work reported in this paper was undertaken for the purpose of testing the pathogenicity for white mice of strains of streptococci isolated from the diphtheria throats.

*Historical.*—Smillie,<sup>7</sup> using Henderson and Palmer's colorimetric method for the hydrogen-ion determination, found 4 strains of hemolytic streptococci isolated from the throats of scarlet fever patients to have a limiting hydrogen-ion concentration of  $P_H$  5.1 to 5.7, all 4 strains were pathogenic for mice; one strain having a limiting hydrogen-ion concentration of  $P_H$  4.5 was not pathogenic for a mouse. Jones<sup>5</sup> isolated 13 strains of hemolytic streptococci from milk, producing a limiting hydrogen-ion concentration of  $P_H$  5.0 to 5.2; five of these were tested as to their pathogenicity for rabbits; the results were negative.

*Technic.*—In the winter of 1921-22 we obtained, through the courtesy of the City Board of Health, Chicago, some fifty Loefflers-serum cultures taken from throats of cases showing a positive B. diphtheria growth. These cultures were obtained a few hours after they had been received at the City Board of Health Laboratory. A platinum loop was used to transfer some of the growth from the Loefflers-serum slants to blood agar plates, otherwise the technic was the same as described in the previous papers.<sup>1</sup> The limiting hydrogen-ion determination was made by the colorimetric method. While the work with these strains of streptococci was in progress, we had, in connection with another problem, the opportunity of using the colorimetric methods of Clark and Lubs,<sup>3</sup> Barnett and Chapmann,<sup>2</sup> Medallia<sup>6</sup> and Gillispie.<sup>4</sup> If the same quality of glassware was used and the same distilled water for all the dilutions, the four methods checked within  $P_H$  0.2 of each other within the limits used for this work. For most of this work the Clark and Lubs method was used.

*Experiments.*—Mice were used to test the pathogenicity of the strains of streptococci. Two mice were used for each strain, one receiving 0.5 c.c., the other 1 c.c. of a 24-hour growth of 1 per cent dextrose hormone bouillon, injected subcutaneously in the back near the tail.

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Twenty strains of hemolytic streptococci from diphtheria throats were used, the limiting hydrogen-ion concentration varying from  $P_H$  4.2 to 5.6. One strain with a limiting H-ion of  $P_H$  4.6 killed the mice with both 0.5 and 1 c.c. injections within 24 hours, one strain with a limiting H-ion concentration of  $P_H$  4.7 with 1 c.c. injection died four days later; the mouse receiving 0.5 c.c. of this strain was not affected. All the other 36 mice were living 6 weeks after the injection. Twenty strains of viridans streptococci from diphtheria throats were injected in the same manner, the limiting H-ion concentration varying from  $P_H$  4.3 to 5.0. All 40 mice were alive 6 weeks later.

Twelve strains of hemolytic streptococci isolated from normal throats with a limiting H-ion concentration of  $P_H$  4.9 to 5.2, and 20 viridans strains from the same source with a limiting H-ion concentration of  $P_H$  4.3 to 5.0, were injected into mice in the same manner as the above diphtheria throat strains. All were negative as to their pathogenicity for mice.

#### SUMMARY

The streptococci isolated from the throats of diphtheria patients do not seem to be pathogenic for white mice. Twenty hemolytic strains, with the limiting hydrogen-ion concentration varying from  $P_H$  4.3 to 5.6 were used, of these only two were virulent for the mice. These two strains had a limiting hydrogen-ion concentration of  $P_H$  4.7 and 4.6 respectively, being midway between the pathogenic or human and the nonpathogenic or bovine types in this respect.

The 20 viridans strains, with a limiting hydrogen-ion concentration varying from  $P_H$  4.2 to 5.0 were used; all were nonpathogenic for mice.

Hemolytic and viridans strains isolated from normal throats were used as controls. Hemolytic strains giving a limiting hydrogen-ion concentration within the human or pathogenic range were selected for this work. All proved to be nonpathogenic for mice.

From the limited number of strains of streptococci, both hemolytic and nonhemolytic, isolated from throats of normal individuals and from diphtheria patients we were unable to find any relationship between the limiting hydrogen-ion concentration and their pathogenicity for white mice.

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## CHANGES IN THE STREPTOCOCCI FLORA OF THE THROAT DURING DIPHTHERIA\*

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WHILE studying the streptococci flora of the throats of children in a State Blind School, during the fall and winter of 1921, a few cases of diphtheria developed. There was a noticeable change in the streptococci of the throats of these cases. In this paper we wish to record the change we found during the period of observation.

*Historical.*—The increase in the number of streptococci found in smears from diphtheria patients' throats is a well-known observation. Loeffler<sup>6</sup> isolated and described streptococci from cases of diphtheria. The first to attach much importance to the streptococci of the diphtheria throat flora were Roux and Yersin, who attributed an increased virulence to the diphtheria bacilli when grown in symbiosis with streptococci. This question has since been studied by several investigators, and the literature has been collected by Bernheim,<sup>3</sup> Le Gros<sup>5</sup> and Ladendorff.<sup>4</sup>

*Technic.*—The technic was practically the same as that used in our former work.<sup>1</sup> The limiting hydrogen-ion concentration was determined according to the method of Avery and Cullen.<sup>2</sup>

*Experiments.*—There were twelve diphtheria and postdiphtheria throats studied; these did not differ markedly from the ones here recorded, but are omitted because the normal streptococci flora of the throat previous to the attack was not ascertained. The accompanying table only contains four of the cases studied, the others were practically the same type of streptococci flora.

*Summary.*—There is a marked increase in number of hemolytic streptococci in the throats of patients during diphtheria. The streptococci in the throat at this time have a limiting hydrogen-ion concentration differing from that of the normal throat flora, this is accompanied by an overgrowth of the types fermenting lactose and salicin. The strains that produce the limiting hydrogen-ion concentration between  $P_H$  4.3 and 4.5 are considered by most investigators as of bovine origin, or nonpathogenic strains, and those reaching the lesser hydrogen-ion concentration limits,  $P_H$  5.0 to 5.5 of human or pathogenic origin. If this is true the streptococci flora of the human throat can under normal conditions be considered as nonpathogenic types and the flora of the diphtheria throats as human or pathogenic types.

The limiting hydrogen-ion concentration must indicate in some way a result of a particular form of metabolic function of the streptococcus. Varia-

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tions in the environment of the organism, causing a change in its metabolism, should change to a certain extent its sensitiveness to a hydrogen-ion concentration.

CASE I						CASE II				
NO. STRAINS										
	LAC.	MAN.	SAL.	H-ION		LAC.	MAN.	SAL.	H-ION	
5 days before exposure	1 B	+	-	+	4.3-4.5	0 B				
	3 Vir	+	-	+	4.3-4.5	2 Vir	+	-	+	4.3-4.5
	3 Vir	-	-	-	4.3-4.5	1 Vir	+	-	-	4.3-4.5
Same day B. Diphtheria were culture from Throat	13 B	+	-	+	5.0-5.4	1 Vir	-	-	-	4.3-4.5
						2 B	+	-	+	5.0-5.3
						1 B	+	-	+	4.3-4.5
14 days after membrane disappeared						2 B	+	-	-	5.0-5.3
	2 B	+	-	+	4.3-4.5					
	4 Vir	+	-	+	4.3-4.5	1 B	+	-	+	4.3-4.5
	1 Vir	-	-	+	4.3-4.5	3 Vir	+	-	-	4.3-4.5
CASE III						CASE IV				
5 days before exposure	1 B	+	-	-	4.3-4.5	0 B				
	1 B	+	-	-	5.0-5.3	3 Vir	+	-	-	4.3-4.5
	1 Vir	+	-	+	4.3-4.5					
	4 Vir	+	-	-	4.3-4.5					
Same day B. Diph- theria were culture from throat	7 B	+	-	+	5.0-5.3	6 B	+	-	+	5.0-5.3
						1 B	+	-	-	5.0-5.3
						0 Vir				
14 days after mem- brane disappeared	1 B	+	-	-	4.3-4.5	0 B				
	2 Vir	+	-	+	4.3-4.5	2 Vir	-	-	-	4.3-4.5
	3 Vir	+	-	-	4.3-4.5	4 Vir	+	-	-	4.3-4.5

B = Beta hemolytic streptococci

Vir = Viridans streptococci

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## DETERIORATION OF PROCAIN SOLUTIONS\*

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### INTRODUCTION

THE extensive use of procain (novocain) as a local anesthetic and as an adjuvant to general anesthesia in major operations warrants an accurate knowledge of any variations in the properties of its solutions when kept over any period of time. It is frequently observed that solutions gradually develop a yellow tinge which deepens with time and is accompanied by a light flocculent sediment usually appearing at first as little tufts or balls, and later assuming a gelatinous appearance: the question immediately arises as to the safe use of such preparations. We inquire therefore:

1. Have there occurred any changes in the anesthetic properties?
2. Has the solution been rendered more, or less, toxic?
3. Is there an increase in the irritability of the preparation?
4. What is the nature of the change in color? May it be an index of a change in the irritability, toxicity, or anesthetic properties?
5. What is the nature of the sediment?
6. Is there any change in the chemical reaction to litmus?
7. Finally, what factors may be responsible for these changes, and how may they be abated?

In this work no investigation of chemical changes was made.

### METHODS AND OBSERVATIONS

One per cent and ten per cent solutions in 0.73 per cent saline were placed in chemically clean bottles as follows: (a) 100 c.c. glass-stoppered bottles, exposed to light. (b) 100 c.c. glass-stoppered bottles, kept in dark. (c) 200 c.c. cork-stoppered Pyrex flasks, exposed to light. (d) 10 c.c. glass (soft) ampules, sealed by heat. A separate ampule was used for each series of determinations. These were the stock solutions with which the repeated examinations were made on the following day intervals: 1, 2, 4, 8, 15, 30, 60, 120, 270. At each determination a freshly prepared 1 per cent solution of procain in 0.73 per cent sodium chloride was used as control.

1. *Anesthetic efficiency.*—Technic of Sollmann† in determining the anesthetic efficiency on motor nerves of the frog was employed. Motor nerves were used because, although more resistant to anesthetic action, they are more reliable than the sensory in their results. The method may be sum-

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†Sollmann, T.: Jour. Phar. and Exp. Ther., 1917, x, 379.

marized briefly as follows: Muscle-nerve preparations were made from the frog's leg to include the lower part of the leg from the knee down, and the entire sciatic nerve from the knee to the spinal cord with a bit of bone attached. The entire nerve was immersed in the anesthetic solution, contained in a little trough cut in a block of paraffin. The excitability was tested with the platinum electrodes of a Harvard induction coil of constant current, applied at the distal end of the nerve at five-minute intervals. The normal efficiency (time required for a freshly prepared 1 per cent solution to cause paralysis) was taken as unity. Any variations in time greater or less than this normal are expressed in fractions of normal efficiency. For example: If the normal requires 15 minutes for complete paralysis, and a specific preparation requires 20 minutes, the efficiency of the latter is 15 divided by 20, or 0.75. In these studies the 10 per cent solutions were accurately diluted to 1 per cent before being applied to the nerve-muscle preparations. Table I shows the results of these observations. Practically no change in anesthetic efficiency occurred in these preparations over a period of nine months, except in the sealed ampules (soft glass) where the efficiency appeared immediately slightly diminished, then remained fairly constant throughout the remainder of the period. Light, darkness, and degree of concentration appeared to have practically no influence on the anesthetic activities of the preparations.

TABLE I  
ANESTHETIC EFFICIENCY

NAME OF PREPARATION	AGE OF SOLUTIONS—IN DAYS								
	1	2	4	8	15	30	60	120	270
1% Procain—Light	1.	1.	1.	0.7	1.	0.8	0.8	1.	0.8
“ —Dark	1.	1.3	1.	1.	1.	1.	0.8	1.2	1.
“ —Flask	1.	1.	1.	0.7	0.7	0.9	1.	1.2	1.
“ —Ampule	0.7		0.6		0.7		1.	0.8	0.8
10% Procain—Light	1.	1.	1.	0.8	0.7	0.8	0.8	1.2	0.8
“ —Dark	0.9	1.2	0.9	0.9	0.9	0.9	1.	0.8	0.8
“ —Flask	0.9	0.8	1.	0.8	0.8	0.8	1.	1.2	0.9
“ —Ampule	0.8		0.8		0.8		1.	0.8	0.8

2. *Toxicity.*—The toxicity was determined by the minimum fatal dose in white mice, injected hypodermically near the base of the tail. Several determinations showed that the toxicity of a 1 per cent solution (freshly prepared) is fairly constant, the minimum fatal dose being eight-tenths mg. of the solid drug per gram weight of the animal. In Table II are shown the minimum lethal doses in terms of milligrams of drug per gram weight of animal. It will be noticed here that there is no distinct change in toxicity that could not be ascribed to variations in the susceptibility of the animals, except in the case of the 10 per cent flask the toxicity of which increased until it doubled. Of this more will be said presently.

3. *Change in Color.*—The most conspicuous change observed was the gradual development of a yellow color in the originally colorless solutions. The degree in depth of color developed was determined by comparison with an empirically prepared caramel standard, “1” being the faintest observable, “100” containing one hundred times as much caramel as “1.” These stand-

TABLE II  
TOXICITY (WHITE MICE)  
MINIMUM FATAL DOSE IN TERMS OF MILLIGRAMS PER GRAM WEIGHT OF ANIMAL

NAME OF PREPARATION	AGE OF SOLUTIONS—IN DAYS								
	1	2	4	8	15	30	60	120	270
1% Procain—Light	0.8				0.8	0.8	1.	1.	0.7
“ —Dark	0.8				0.7	0.6	0.8	0.8	1.
“ —Flask	0.8		0.8	0.6	0.6	0.6	0.8	0.9	0.8
“ —Ampule	0.7		0.7		0.6		0.6	0.6	0.6
10% Procain—Light	0.8			0.8	0.8	1.	0.8	0.8	1.
“ —Dark	0.8			1.	0.8	1.	1.2	1.	0.8
“ —Flask	0.8	0.8	0.7	0.8	0.7	0.6	0.5	0.4	0.4
“ —Ampule	0.8		0.9		0.8		0.7	0.6	0.7

ards were made up but once, and kept as permanent standards for comparison. Table III shows the results. It will be observed that at the end of four months there developed in some of the solutions a brown tinge which somewhat obscured the readings of the caramel standard. These color changes, as indicated by comparing Table III with Tables I and II, has apparently no relation to the anesthetic and toxic properties of the solutions, with the exception of the 10 per cent flask. Here we find a marked color change, with a doubling of the toxicity. The stopper of this flask was accidentally removed for a few days during the first month of the test. Increased contamination from the dust of the room may be offered as an explanation for this toxicity, since it is to be noted that the sealed ampules showed very slight development of color.

TABLE III  
CHANGES IN DEPTH OF COLOR—YELLOW

NAME OF PREPARATION	AGE OF SOLUTIONS—IN DAYS								
	1	2	4	8	15	30	60	120	270
1% Procain—Light	0	0	0	1	2	6	30	30	30*
“ —Dark	0	0	0	1	1	6	8	8	24
“ —Flask	0	0	0	2	3	8	24	24*	24*
“ —Ampule			1		2		1	1	150*
10% Procain—Light	1	1	1	5	5	5	8	16	2
“ —Dark	1	1	1	4	5	5	8	16	200*
“ —Flask	1	1	1	5	5	6	10	24*	300*
“ —Ampule			5		4		4	5	5

\*These solutions developed a brown tinge which obscured the yellow color. The depth of the tinge was about proportionate to the figures above indicated.

4. *Irritability.*—The irritability was determined by dropping 1 per cent concentrations of the solutions into the eye of a rabbit, and confirmed like solutions into the human eye. At no time did the rabbit's eye show appreciable irritation. The human eye was distinctly irritated by all the solutions that had developed the brown tinge, but not by the others.

5. *Appearance and Nature of the Sediment.*—After standing four days at room temperature a very faint flocculent sediment, not unlike tiny tufts of cotton less than one centimeter in diameter, appeared in all the bottles, but none whatever in any of the ampules. The tufts were distinctly smaller in the 10 per cent solutions. This sediment increased gradually in quantity and assumed a rather gelatinous appearance. When removed with a wire loop the mass clung together, and reminded one of “mother of vinegar.”



Microscopic examination showed the mass to be the mycelia of common bread mold. To determine whether or not the bread mold was alone responsible for this sediment, four hard glass Florence flasks containing 1 per cent procain in 0.73 per cent saline were prepared, for comparison, as follows: The contents of three of the flasks were sterilized by boiling five minutes on three successive days, and making up the loss with sterile distilled water. One flask was stoppered with sterile plugging cotton, one with cork, and one with a rubber stopper. The fourth flask was prepared by adding to a flask of sterile 0.73 per cent saline sufficient procain to make 1 per cent (weighed and added under aseptic precautions), and stoppered with a sterile rubber stopper. Over a period of six months, at room temperature, there appeared no sediment in the three flasks. The color changes were a distinct stair-step phenomenon, the solution in the cotton-stoppered flask was a decided yellow, in the rubber-stoppered flask only a faint yellow tinge, and in the cork-stoppered flask intermediate. There was no suggestion of a brown coloration, even after a period of twelve months. There was no change in the anesthetic, toxic, and irritability characteristics of the solutions as determined by the methods outlined heretofore. The fourth flask showed the characteristic sediment of bread-mold in a coffee brown solution. This brown solution was distinctly irritating, and slightly more toxic than the other preparations. These observations indicate to us that the sediment is due to the growth of "molds" in the solution. These molds have apparently no influence on the irritability, toxicity, or anesthetic properties of the solutions until the appearance of the distinctly brown coloration. Moreover, the development of yellow color appears to be due to some oxidative process, as the greater the access of air (example: the cotton-stoppered flask) the deeper the color change, while with the almost total occlusion of air (see ampule, Table III—Color change) there was practically no change in color.

6. *Reaction*.—All the preparations, with the exception of the ampules, were faintly acid to litmus until the development of the brown coloration, when the reaction changed slowly from faintly acid to faintly alkaline. The ampules were uniformly very faintly acid or neutral.

#### CONCLUSIONS

1. There is practically no deterioration in the anesthetic properties of procain solutions standing over a period of several months.

2. There is no increase in toxicity or irritability except in case of gross contamination.

3. The yellow color change is no index of a change in anesthetic, irritating, or toxic properties. The development of this yellow color appears to be due to an oxidative process.

4. The sediment in procain solutions may be due to the growth of molds. Their spores may be present in the original preparations. They indicate "contamination" long before an increase in irritability and toxicity develops.

5. Procain solutions are therefore stable. If kept sterile and out of contact with air they will remain unchanged indefinitely.

# LABORATORY METHODS

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## A SIMPLIFIED TECHNIC FOR CLINICAL BLOOD CHEMISTRY\*

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THANKS to the splendid work of Folin, Van Slyke, Marshal, Benedict and others, the chemical analysis of the blood, or, as it is called more briefly but less correctly, blood chemistry is no longer today what it used to be 15 or 20 years ago. Much less time is consumed and much less blood is required, and the results are more accurate and dependable. Blood chemistry is now part of the routine work in most hospital laboratories and is of recognized value both in medical and surgical treatment.

In view of the growing popularity of blood chemistry, it appeared to us that the technic could be simplified still more. In fact, simplification is anything but a freak when it is realized that in the Beth Israel Hospital Laboratory, where our work has been carried out, from twelve to fifteen specimens are analyzed on the so-called "blood chemistry days," and the routine analysis includes  $\text{CO}_2$  combining power, urea nitrogen, nonprotein nitrogen, creatinin, uric acid, cholesterol and sugar. The accuracy was by no means sacrificed for the sake of simplification, as several hundreds of comparative tests showed, when the standard and the simplified technic were used simultaneously. Several details appeared to us entirely unnecessary, although they are generally accepted as essential parts of the method.

We could not see the necessity of adding potassium sulphate and copper sulphate when determining nonprotein nitrogen. They are necessary in the original Kjeldahl process, when much larger quantities are used for analysis. There, they shorten considerably the time of reaction. But we don't need catalyzers or temperature raisers for the destruction of the minute quantities of non-protein nitrogenous compounds which are present in about  $\frac{1}{2}$  c.c. of blood. Potassium sulphate and copper sulphate may contain nitrogen and thus become a source of error. It is true that the error is small, as only very small quantities of both substances are used, but the larger the number of substances used in the analytical process, the larger the sum of errors.

To our mind, an ideal method is one which calls for a minimum number of reagents, a minimum number of glassware and a minimum amount of manipulation. We had all these three requirements in mind when we were developing our simplified technic.

Whatever the number of reagents, their purity, at least their freedom

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from substances which may affect the accuracy, is of the utmost importance. This is obvious in the case of the determination of urea and nonprotein nitrogen as ammonium salts may occur in many inorganic compounds.

We use the following substances in the determination of urea and non-protein nitrogen: Hydrochloric acid, sulphuric acid, sodium tungstate, sodium hydroxide, potassium hydroxide, hydrogen peroxide, potassium iodide and mercuric iodide. We found that all of them will give often a distinct reaction with Nessler's solution, sufficient to affect materially the result of the analysis, despite the fact that they are labelled C.P.

It would be unfair to call it a misrepresentation, as the amount of ammonium salts is entirely negligible for the many purposes for which the C.P. chemicals are prepared; purposes which do not include blood chemistry. The requirements of blood chemistry are too small to justify an additional outlay in getting the chemicals used in the blood analytical process absolutely free from ammonia. We therefore do our own purifying of the reagents, with the exception of sulphuric acid since we don't know of any way of freeing the latter from ammonium salts. But, whether the reagents be free from nitrogen or contaminated with the latter, a blank test must always be made along with the determination of the constituents of the blood. This is to ascertain to what extent the reagents are free from contaminations affecting the accuracy of the analysis. The freedom should never be taken as a matter of course.

The blood chemistry is being carried out at present by so-called "technicians," i.e., persons with very meagre chemical knowledge or none at all. It is therefore important to simplify the technic from one side and from the other side to emphasize many details with which every analytical chemist is familiar and which are indispensable when accurate results are to be expected.

Among these details are scrupulous cleanliness and careful, intelligent preparing and handling of the standard solutions. It is not so important for a technician to know just how many methods there are for the quantitative determinations of sugar in blood and what are the respective advantages of each one, as it is to know how to carry out a test according to one with least expenditure of time and the greatest accuracy; the latter from the clinician's standpoint.

Blood chemistry is, after all, nothing but a part of the analytical chemistry and the laws and rules of the latter are applicable to it also. Whether the ammonia, formed as a result of saponification of urea or destruction of the nonprotein nitrogen constituents of the blood, is distilled over or aerated, or whether either operation is omitted and the Nesslerization is carried out directly, is not so important as whether all the requirements of quantitative analysis have been complied with.

Duboseq's colorimeter may have certain advantages over Hellige's or vice versa, but the accuracy of the results depends to a much larger extent upon the purity of the chemicals used as standards than upon the kind of colorimeter. The testing of ammonium sulphate, potassium dichromate and

other standard reagents for their purity should be known to every blood chemistry worker.

Until the advent of the urease method, several methods of urea determination were in vogue, Folin's method, decomposition of urea with sodium hypobromite, Bang's, etc. The second, the hypobromite method, was notoriously incorrect; the first was capable of yielding correct results. Yet all the methods of urea determination got into disrepute and many advocated instead, the nonprotein nitrogen determination because the quantitative analysis of blood was done chiefly by persons not skilled in chemistry and a perfectly good method was full of traps for them.

This must be outspoken because the blood chemistry is a comparatively new thing with a big future; its field is not the chemical but the hospital laboratory, its cultivators are not the professors of pathology and biochemistry but clinicians, whose training was accomplished along different lines from those of chemistry, and their assistants, the so-called technicians. They should, therefore, be supplied with a technic which is simple and at the same time does not assume too much in the matter of fact.

Our article is a feeble effort in that direction, and at the same time, an effort towards standardization of the technic, elimination of the many methods and general acceptance of one. It would be no exaggeration to say that every hospital laboratory uses a different method of blood chemistry. This allows of no comparison between the findings of two institutions on the same subject.

To Folin, we owe an accurate method of determining urea, nonprotein nitrogen, uric acid, sugar and creatinin in 5 c.c. of blood. Many dreadful things were said against the microchemical methods and many lances were broken for the macrochemical ones. Yet no amount of praise could save the latter. Blood is not urine, and it is desirable to take as little blood from the patient as possible. It is the more desirable, when the blood taking has to be done repeatedly. It is of no use to take 25 c.c. when 5 c.c. will suffice. Sometimes, it is difficult to get more than a small volume. This applies to adults as well as to children. An analysis may go wrong, and it is necessary to repeat it, or it may be desirable to check up results.

We have found that 7 c.c. of well-oxalated blood are sufficient for an analysis which includes  $\text{CO}_2$  combining power, urea, nonprotein nitrogen, creatinin, uric acid, cholesterol, sugar and chlorides. By well oxalated blood, we understand blood which is free from clots. It is obtained not by using plenty of oxalate, but by shaking the blood thoroughly immediately after it has been poured into the tube. As to the amount of oxalate to be used in order to prevent coagulation, we have found that 0.05 gms. is quite sufficient for 5-15 c.c. of blood. It is very convenient to introduce 1 c.c. of a solution of potassium oxalate into a test tube, to allow the liquid to wet the inside of the tube and dry it in the sterilizer. But, even with such a "prepared" tube, the shaking of the latter immediately after it has been filled with blood must not be omitted.

The technician has to analyze very often several specimens of blood



and when a more or less complete examination is required, it is desirable to do it according to a certain system which eliminates waste of time and with the smallest amount of equipment. In our work, we use nothing but glass tubes, plain and calibrated, the calibration being done by ourselves. No flasks, measuring or otherwise, funnels plain or separatory, are used. With one exception, the filtering is done away with. Whole blood is used in every test. Only in the case of  $\text{CO}_2$  combining power do we use the oxalated plasma because the whole blood would smear the apparatus and make cleaning difficult.

We begin the analysis of several specimens by arranging eight rows of glass tubes. The first row, which we will call A, contains calibrated tubes marked 5, 10, and 15 c.c. The second row, B, contains calibrated tubes with one 10 c.c. mark. The third and fifth rows, C and E, are made up of plain test tubes, not necessarily dry, and the fourth, sixth and eighth rows, D, F, and H contain plain dry test tubes. The seventh row, G, contains dry test tubes calibrated to the 25 c.c. mark.

All the tubes are washed with an alkaline soap solution and thoroughly rinsed, first with tap and then with distilled water.

Blood specimens are centrifuged for about 8 to 10 minutes. Two c.c. of the clear oxalated plasma are pipetted off from each specimen and transferred to the tubes in row G which are then closed with cotton plugs and put away in the ice box until such time as one is ready to start with the determination of  $\text{CO}_2$  combining power and cholesterol. The remaining 5 c.c. of blood are distributed between rows A and B, A getting 3 c.c. and B 2 c.c. The 3 c.c. are for the determination of urea, nonprotein nitrogen and uric acid. The 2 c.c. are for the determination of creatinin, sugar and chlorides.

The number of tubes in each row (row A excepted) is equal to the number of specimens to be analyzed. Row A has an extra tube which serves to control the efficiency of the urease solution and is therefore marked K.

To each blood-containing test tube in row A, we add 2 c.c. of water. Tube K gets 4 c.c. of water and 1 c.c. of a standard urea solution (1 gm. urea in 1000 c.c. of distilled water). We add to each tube 1 c.c. of urease solution prepared in the following way: 1 c.c. of permutit is washed once with 3 c.c. of 2 per cent acetic acid, and three times with distilled water, using 15 c.c. each time. The washing is done most conveniently in a capacious centrifuge tube, the water being decanted each time. Add 20 c.c. of 14 per cent alcohol, 3 c.c. of 95 per cent alcohol, 17 c.c. of water and close with a clean stopper. Shake gently for about 5 minutes, then centrifuge. This is, in the main features, Folin's proposed method for the preparation of urease solution as described by him in the *Journal of Biological Chemistry*, (1916, xxvi, 501 and 1919, xxxviii, 111).

We only reduced the quantities to one-fifth. The advantage is twofold. We avoid filtering and can use freshly prepared solutions since the preparation of the latter takes about 15 minutes.

After the urease has been added to each of the tubes in row A, the rack is put into a water-bath which has a temperature of  $50^\circ \text{C}$ . and kept

there for about five or ten minutes. It is very important not to let the temperature rise over  $50^{\circ}\text{C}$ . An appreciable coagulation of the protein can be noticed at even  $55^{\circ}\text{C}$ . and it increases as the temperature rises. The coagulation prevents part of the urea from being acted upon by the urease.

The rack is now taken out of the water-bath and allowed to cool. We turn our attention to row B, each tube of which we fill with distilled water up to the mark. The water is allowed to run along the walls of the tube in order to avoid foaming as much as possible. When foam is present, the addition of water should stop when the liquid is just underneath the meniscus. A drop or two of ether, amyl alcohol, or grain alcohol is added and this is sufficient to break up whatever foam there is on the surface. The tube is now filled up to the mark with a few more drops of distilled water and a pinch of dry picric acid is added. With a clean glass rod, we work the picric acid into the diluted blood until the liquid has assumed a dirty yellow color. We take out the glass rods and allow the tubes to stand a while.

The tubes in row A have been cooled down to room temperature. We add to each one 4.5 c.c. of 10 per cent sodium tungstate and fill up to the mark with  $2/3$  N. sulphuric acid observing the same precautions to avoid foaming and using the same means to break up the foam as in the case of row B. Folin uses 5 c.c. of sodium tungstate and 5 c.c. of  $2/3$  N. sulphuric acid in order to deproteinize 5 c.c. of blood. We found this proportion insufficient when whole blood is used. The  $2/3$  N. sulphuric acid is about 3.3 per cent and can be prepared approximately by adding slowly 18 c.c. of concentrated sulphuric acid to about 800 c.c. of distilled water in a 1000 c.c. volumetric flask and making up to the mark.

The liquids in the tubes of row A are stirred with clean glass rods until the red color of the blood has turned to dirty brown. The rods are now taken out and the tubes in both rows centrifuged for 10 to 15 minutes. While the tubes are being centrifuged, the apparatus for aeration is set up. It is made up of a copper rack with two rows of test tubes. The test tubes are of heavy glass. Those of one row are graduated into 1 c.c. divisions up to 100 c.c.; those of the opposite row are plain. Each tube is equipped with a double perforated rubber stopper with two glass tubes. One ends  $1/2$  inch underneath the stopper, the other one  $1/4$  inch above the bottom of the test tube. Both protrude about two inches above the stopper. When making the holes in the stopper, one has to use a borer which is a size smaller than the tube, so that the latter will pass with some difficulty. (A clean, smooth hole in a rubber stopper is made easily by wetting the borer with 5 per cent lye and holding the stopper firmly against a solid surface while boring.) As soon as the tube begins to move loosely through the stopper, the latter is to be rejected.

Each row begins with a short glass tube and ends with a long one, and vice versa. Each short glass tube of one row is faced by a similar one in the opposite row and vice versa.

The test tubes of both rows and the glass tubes are carefully washed with soap and rinsed thoroughly first with tap and then with distilled

water. The graduated tubes are now filled with 10 c.c. of approximately N/30 hydrochloric acid (997 of distilled water, 3 c.c. of fuming hydrochloric acid) and closed tightly with the rubber stoppers and the rubber tubes attached. The plain tubes are loosely closed with the rubber stoppers in such a way that the short glass tubes are outside of the test tubes.

Having proceeded thus far with the preparations for aeration, we now start with the oxidation of the nonprotein nitrogenous constituents of the blood. We set up a row of tiny Erlenmeyer flasks, 30 to 40 c.c. in capacity. We draw off with a clean dry pipette 8 c.c. of the clear supernatant liquid from each of the tubes of row A and run 3 c.c. into each of the tiny Erlenmeyer flasks and 5 c.c. into each of the tubes of row E. Another 3 c.c. portion of the filtrates, which may be turbid, is introduced into each of the plain aeration tubes marked "U." We then take an extra Erlenmeyer flask and run in 0.9 c.c. of 10 per cent sodium tungstate and 0.9 c.c. of 2/3 N. sulphuric acid of urease and 1 c.c. of distilled water. This flask will serve as a blank test, that is, it will indicate how much nitrogen there is in all the quantities of the chemicals used in the determination of urea N. and nonprotein nitrogen.

It may appear that it would be a much simpler procedure to determine once for all the degree of freedom from objectionable compounds of all the chemicals used in the determination of urea N. and nonprotein nitrogen. We can deduct the sum of the quantities of nitrogen combined in the quantities of the chemicals used in the analysis from the quantity of the found nonprotein nitrogen and thus eliminate the error due to contamination. Yet this would not be correct. Occasional carelessness on the part of the operator, or tampering with by unqualified persons may bring about a contamination after the chemicals have been tested and the degree of their purity, so far as the nitrogen is concerned, duly determined. We cannot say enough to emphasize the importance of a blank test in microchemical blood analysis. We will go so far as to declare as doubtful any analysis where a blank test has been omitted. The use of a blank test where it has been previously omitted, will sometimes produce very curious results. It will bring high urea nitrogen and nonprotein nitrogen figures where they are inexplicable within acknowledgingly normal limits and eliminate a good deal of speculation.

Some observers found that normal persons may have as much as 27 mg. of urea nitrogen in 100 c.c. of blood. It would be curious to know whether they used a blank test and in what other way they made it certain that all of the nitrogen they found originated in the blood.

To go back to our Erlenmeyer flasks, we run into each one  $\frac{1}{2}$  c.c. of concentrated sulphuric acid, put the flasks upon a sand bath and the latter upon the electric stove. The boiling proceeds very smoothly and without bumping. If there is such annoyance, the layer of sand is made a bit thicker. After the liquid has turned a black-brown, the heating is still continued for about five minutes, after which the flasks are taken off the sand bath and allowed to cool.

After the flasks have cooled, one may add to each 1 c.c. of hydrogen

peroxide if one wishes, but this is not necessary. The hydrogen peroxide merely burns off the carbon and restores to the liquid its original color, but it does not complete the conversion of the organic nitrogen into ammonia. This job is thoroughly done by sulphuric acid. The use of hydrogen peroxide would be a necessity if it were possible to Nesslerize directly without previous aeration.

Unfortunately, the direct Nesslerization fails so often or yields solutions which turn rapidly turbid, that we do not advise its use at all. To be sure, putting up the apparatus for aeration and disconnecting it after the aeration is a considerable annoyance. But an even greater annoyance is noticing how the liquid which has been Nesslerized rapidly turns turbid and a red sediment begins to fill up the tube and makes the determination impossible.

Despite numerous efforts, we are unable to explain what causes the precipitation of the red sediment. Having noticed that the direct Nesslerization of the liquid containing ammonia, as derived from a standard urea solution, incubated with urease, gave less trouble, we thought that the latter might be due to the presence of an alkali sulphate. (Alkali sulphate is formed when Nessler's reagent is added to sulphuric acid.) We added variable quantities of potassium and sodium sulphate to standard ammonium sulphate and then added Nessler's solution. We had no trouble in Nesslerizing. This shows that the alkali sulphates are not responsible for the turbidity. We tried a mixture of phosphoric and sulphuric acids as suggested by Folin. The results were not encouraging and the mixture attacked the flasks. We did not give up hope yet of finding the conditions under which a Nesslerized liquid will keep for several hours as is the case when aeration has been used. But for the present, we prefer not to take any chances with direct Nesslerization.

While the Erlenmeyer flasks are being heated on the electric stove, we may start with the determination of the  $\text{CO}_2$  combining power. The procedure is similar to the one described by Van Slyke. Yet we thought it expedient to introduce a few changes.

Instead of separatory funnels we use plain dry test tubes (row F) large enough to accommodate a double perforated stopper. The latter has two glass tubes passing through it. One is short and ends underneath the stopper. The other one is longer and ends  $\frac{1}{4}$ " above the surface of the blood. Each tube is in its turn closed with the stopper, air is blown through which has previously passed a bottle filled partly with glass beads and 1 c.c. is introduced into the  $\text{CO}_2$  apparatus which has been previously evacuated in the well-known way. For convenience, the stem to which the  $\text{CO}_2$  apparatus is attached is equipped with three bottles having syphons. One bottle is filled with distilled water, the other one with caprylic acid, the third with 5 percent sulphuric acid. This allows quick washing of the apparatus previous to evacuation and obviates the necessity of testing for the presence of acid with ammonia.

It is customary to manipulate the apparatus after sulphuric acid has been



added so that the mixture of blood water and sulphuric acid is finally brought to the larger knee of the  $\text{CO}_2$  apparatus while the mercury is allowed to rush into the calibrated part through the more narrow knee. This is not necessary. There is little difference whether the gas touches mercury or the mixture of the liquids—the result is not affected by this fact materially. Why then the manipulation? Furthermore, the apparatus could be made by entirely omitting the more narrow knee and it would be less fragile and expensive.

The aeration can now be started. The contents are transferred carefully to the plain aeration tubes marked NPN, the flasks rinsed twice with 2 or 3 c.c. distilled water. Every plain tube, whether marked NPN or U gets enough sodium hydroxide (20 per cent) to produce a distinctly alkaline reaction, and enough amylalcohol to form a layer about one-fourth inch high. There is no reason to fear that a loss of liberated ammonia will follow the addition of lye if the aeration tubes are not closed quickly. We cannot see how such loss could take place, as we are using only 1.2 c.c. of blood for both urea N. and NPN determination.

When all the tubes are closed with stoppers they are connected with the suction pump and the latter turned on full. Thirty minutes are sufficient to complete the aeration. We loosen the stoppers beginning with the tube connected with the sulphuric acid bottle, letting the water run through the suction pump in the meantime. When the last stopper has been loosened, we shut off the water, pull out each stopper and rinse the long glass tube of each stopper with distilled water. We run 2 c.c. of an ammonium sulphate solution containing 0.944 gm. of  $(\text{NH}_4)_2\text{SO}_4$  in 1000 c.c. water into a 25 c.c. graduated cylinder, then 10 c.c. water, then 8 c.c. diluted Nessler's solution (1 part Nessler's solution, 5 parts water). The color thus produced is compared in Hellige's colorimeter with the color obtained when the diluted Nessler's solution is added gradually to each of the tubes containing ammonium chloride and the tubes are then filled up to the 20 c.c. mark. In case of large quantity of urea in the blood, we dilute to 40, 60, 80 or 100 c.c.

*Calculation with the Hellige Colorimeter.*—Suppose the reading in the case of urea was 25. Multiplying this number by .0225 we find the number of mg. of nitrogen which 100 c.c. of a solution must contain in order to produce the same intensity of color. It will be 0.56 mg. It is obvious that 20 c.c.

contain only  $\frac{0.56 \times 20}{100} = \frac{0.56}{5}$  mg. For the estimation of urea nitrogen, we took 3 c.c. of blood diluted to 15 c.c. and then pipetted off 3 c.c. These 3 c.c. represent  $\frac{3 \times 3}{15}$  or  $\frac{3}{5}$  c.c. of the blood. One hundred c.c. of the blood will

contain  $\frac{0.56 \times 5 \times 100}{3 \times 5} = \frac{56}{3}$  mg. urea nitrogen, or urea nitrogen =  $\frac{25 \times 2.75}{3} = 25 \times \frac{3}{4}$

Assuming that:

R- Reading (Number of mg. corresponds to number read on scale of colorimeter.)

A- Number of c.c. of blood taken for analysis

V- Volume to which the blood was diluted

b- The number of aerated and subsequently Nesslerized c.c. of clear filtrate

V<sub>1</sub>- Volume to which the Nesslerized solution was diluted

we get the following general formula:

$$\text{Urea N} = \frac{R \times .0225 \times V \times 100 \times V_1}{ab \times 100} = \frac{R \times .0225 \times V \times V_1}{ab}$$

The same formula applies to the calculation of nonprotein nitrogen.

We are now ready to determine glucose, creatinin and the chlorides.

We draw off about 5.5 c.c. of the supernatant yellow liquid from each of the tubes in row B and run in 2 c.c. into each of the tubes in row D, 3 c.c. into each of the Erlenmeyer flasks in row K and 0.4 c.c. into each of the tubes of row E. We add to each tube in row D 0.1 c.c. of 10 per cent NaOH or, still better, 0.1 c.c. of 2 N. sodium hydroxide. We mix well and allow ten minutes for the reaction between the creatinin, sodium hydroxide and picric acid. The reaction is supposed to be a reduction, the picric being reduced in alkaline solution to picramic acid. It is also possible that some kind of an azo-oxy compound is formed. Since azo-oxy compounds are not very stable and change readily into azo compounds, it is not recommended to wait longer than 10 to 15 minutes. During this time, we add  $\frac{1}{2}$  c.c. of 10 per cent sodium carbonate to each of the test tubes in row C and heat. The yellow liquid begins to turn red. When there is no more perceptible change in color, the heating is discontinued.

We now fill up the wedge of Hellige's colorimeter with a N/4 solution of potassium dichromate (12.44 gm. in 1000 c.c.), pour successively the contents of each of the tubes of row D into the small cup of the colorimeter and take a reading.

*Calculation.*—The number of mg. of creatinin per 100 c.c. of blood is found by multiplying the number found through matching the standard with the unknown by 5 (as the blood has been diluted 5 times) and 100.

The contents of each of the tubes in row C we pour successively into the graduated tube of Kuttner's colorimeter, rinse the tube out a few times with a few drops of water, and then add so much water until the color matches with that of either standard A or B (the latter is twice as strong as A). The mark on the graduated tube to which the liquid had to be diluted gives automatically the number of mg. of glucose per 100 c.c. of blood if A has been used. When B is used, we multiply the number by 2. When strongly diabetic blood is to be used, we use 0.2 c.c. instead of 0.4 c.c. of the yellow liquid.

We found Kuttner's colorimeter as reliable in *clinical* work as other colorimeters, and a good deal more convenient in view of the minute quantities of blood and little time required to carry out its determination. We wish

to state, however, that the blood should be drawn from an arm vein and not by finger puncture to obviate dilution by tissue juices.

*Chlorides.*—To each Erlenmeyer flask of row K, we add 10 c.c. of n/50 silver nitrate, which also contains ferric ammonium sulphate. (This ammonium sulphate-silver nitrate solution is best prepared by dissolving 2.158 gm. of silver nitrate in 600 c.c. of distilled water contained in a 1000 c.c. measuring flask, adding a solution of 100 gm. ferric ammonium sulphate in 100 c.c. of 25 per cent nitric acid which have been previously boiled in order to expel the nitrous acid, and then filling up to the mark with distilled water.) The contents of the Erlenmeyer flasks are stirred well and heated. The silver chloride will coagulate and will not interfere with the further course of the analysis. We now add 5 c.c. of distilled water and titrate with n/50 ammonium sulphocyanate. When all the silver has been precipitated as insoluble silver sulphocyanate, the reaction between ammonium sulphocyanate and the ferric salt, characterized by a red color, will take place.

*Calculation.*—Suppose the excess of silver nitrate was 7. This means that 3 c.c. of n/10 silver nitrate were necessary to precipitate the sodium chloride present and that the 3 c.c. which we took for the determination of the chlorides contained as much sodium chloride as there is in 3 c.c. of a n/50 sodium chloride solution. n/50 sodium chloride solution contains 1.169 gm. per liter or 1.17 mg. in 1 c.c.. Three c.c. will contain 3.51 mg. of sodium chloride, and 1 c.c. of blood will contain  $3.51 \times \frac{10}{3.2}$ . One hundred c.c. will contain  $3.51 \times \frac{10}{3.2} \times 100 = .585$  mg. of sodium chloride.

$$\text{Sodium chloride (100 c.c. of blood)} = (10-7) \times 1.17 \times \frac{10}{2 \times 3} \times 100$$

$$\begin{array}{l} \text{If we put } 7 = a \\ \quad \quad 3 = b \\ \quad \quad 2 = c \end{array}$$

we obtain the following general formula:

$$\text{NaCl (100 c.c. blood)} = (10-a) \times \frac{1170}{bc}$$

*Uric Acid.*—Into each of the tubes in row E. we run 2 c.c. of a 5 per cent silver lactate solution in 5 per cent lactic acid. This precipitates with uric acid as silver urate. The tubes are now centrifuged for about four minutes, the clear supernatant liquid poured off, 5 c.c. of water added, the precipitate stirred up, the centrifugation and the subsequent decantation repeated. We now add just enough of a 5 per cent sodium cyanide solution to dissolve the precipitate. We transfer the liquid to a 10 c.c. graduated cylinder, wash out twice with a few c.c. of a saturated sodium carbonate solution (225 gm. in 1000 c.c. of water). We now prepare the standard by running into a 50 c.c. volumetric flask 10 c.c. of the standard uric acid solution, prepared according to Folin. (1 gm. of uric acid dissolved in 500 c.c. of water with the aid of 1 gm. lithium carbonate; 50 c.c. are transferred to a 1000 c.c. measuring flask, 500 c.c. of a 20 per cent solution of sodium sulphite are added, the flask is filled up to the mark and the uric acid solution

transferred to 200 c.c. bottles which are kept tightly stoppered.) The 10 c.c. of standard uric acid solution represents 1 mg. of uric acid. We now add 20 c.c. of saturated sodium carbonate solution and 1 c.c. of uric acid reagent, prepared according to Folin, fill up to the mark with distilled water, mix well and run the blue liquid into the wedge of Hellige's colorimeter. To the liquid in the 10 c.c. graduated cylinder, we add  $\frac{1}{2}$  c.c. of uric acid reagent, fill up to 5 c.c. and mix well before taking reading.

*Calculation—*

$$\text{U.A. (100 c.c. blood)} = \frac{R \times V \times V_1 \times 100}{ab \times 100} = \frac{R \times V \times V_1}{ab}$$

R = Reading (By reading we understand the number of mg. corresponding to the number read on the scale of the colorimeter.)

V = Volume to which blood has been diluted

$V_1$  = Volume to which blue liquid has been diluted

a = Number of c.c. of protein free filtrate taken for uric acid determination

b = Number of c.c. of blood used for the determination of urea N., NPN, and uric acid

Like several other observers, we noticed that if the sodium carbonate solution is added before the uric acid reagent, the blue color does not appear often. We can offer no satisfactory explanation for this phenomenon.

*Cholesterol.*—To each of the tubes in row G, we add 10 c.c. of alcohol-ether mixture (3 parts absolute alcohol to 1 part ether) and run in  $\frac{1}{2}$  c.c. of blood very slowly, drop after drop, stirring well with a glass rod after every addition. We heat the tubes until the liquid begins to boil, cool to room temperature, take out the rods, rinse them with little of ether-alcohol mixture, fill up with this liquid till the mark, filter into dry clean tubes (row H), draw off 10 c.c., run into dry clean 30 c.c. beakers and evaporate the ether-alcohol mixture in a water-bath of about 85° C., taking care that the heating should be discontinued as soon as the beakers are dry. We extract the cholesterol three times with small portions of warm cholesterol (1 c.c. at a time), transferring the extract to a graduated 10 c.c. cylinder, fill up to the 1 c.c. mark with chloroform, add 2 c.c. acetic anhydride (the latter must be colorless—if it became colored, it must be rectified by using an air condenser) and one drop of concentrated sulphuric acid and shake. It is customary to put the mixture in a dark place and wait ten minutes until the full color develops, which is then compared with a standard solution of naphthol green B. (2 c.c. of a 0.1 per cent aqueous of the dye with 17 c.c. of water). The 0.1 per cent solution is good for about a month, after which time it should be rejected and a fresh one prepared. When freshly prepared, it should be checked up by a standard cholesterol solution, although the necessary correction is mostly within the limits of permissible error.

*Calculation —*

$$\text{Cholesterol (100 c.c. blood)} = \frac{R \times V \times 100}{B \times A}$$

R— Reading

B— Amount of blood used

V— Volume to which the alcohol-ether extract has been diluted

A— Amount of alcohol-ether extract taken for determination.

We wish to thank Dr. Max Kahn for his friendly advice during the course of these experiments.



## A SUCCESSFUL METHOD FOR THE ISOLATION OF DIPHTHERIA BACILLI FROM MIXED CULTURES\*

BY ANNA I. VAN SAUN AND ISABELLE A. GRAVES

IT would seem as though the last word must have been said long ago with regard to cultural work on diphtheria. Conditions vary so in different places however, that methods which are excellent for one laboratory are quite impossible for another and it has occurred to us that the results of our experiences with various procedures for use in the isolation of the diphtheria bacillus when neither ascitic fluid nor blood could easily be obtained, might be helpful to other laboratories in the same position.

We communicated with a number of laboratories as to methods used and discovered that several of them varied as to their plating medium.

One large State Laboratory, that of New York, plates the original culture on dextrose serum agar and also uses ascitic broth for enrichment. Fishings are made to Loeffler's. This method has been in use for a number of years in the Research Laboratory of the Department of Health of New York City and was first suggested by Dr. Anna Williams. We also used this method whenever we could obtain ascitic fluid. It gives excellent results.

The State Laboratory of Connecticut has followed the above method whenever ascitic fluid could be obtained.

The State Laboratory of Massachusetts has used large tubes of Loeffler's blood serum for the isolation of the diphtheria bacillus, making a number of transplants from the original culture and fishing with the aid of a hand lens from typical colonies. The director states that good results have been secured by the use of this method.

The Laboratory of the City of Chicago sends out an outfit consisting of a small aluminum box filled with Loeffler's blood serum which really constitutes a small plate and with which good results are said to be obtained. We have not personally tried this method owing to considerable difficulty experienced in handling the boxes.

The Laboratory of the City of Bridgeport uses tubes of Loeffler's in the manner of the State Laboratory of Massachusetts and fishes to broth. The Director of the laboratory states that the method is not successful with contaminated or overgrown cultures. We have not been successful in growing the diphtheria bacillus in plain broth and therefore did not try the above method.

The Bender Hygienic Laboratory of Albany, N. Y., plates the original culture on blood agar, fishing from thence to Loeffler's blood serum. The Director informs us that the results are excellent. We were not able to use this method on account of the difficulty in obtaining blood. Horse serum agar was

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\*From the Bureau of Laboratories, Department of Health, New Haven, Connecticut.  
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suggested but it was found that the cost of sterile horse serum to any but anti-toxin laboratories would be prohibitive.

It seemed to us that the method used by Miss Beckler of the State Laboratory of Massachusetts was the simplest of those noted above and we tried it, but our experience with it led us to believe that a larger inoculation surface would be an improvement, especially with badly contaminated or overgrown primary cultures. We therefore experimented with Loeffler's blood serum in Petri dishes, finally securing plates which gave excellent results with 90 per cent of our cultures. The laboratory runs a census of about twelve thousand (12,000) diphtheria cultures annually—5765 were received from January 1, 1922 to July 1, 1922. One hundred and forty-five (145) cultures were selected from this number for isolation.

Any good formula for Loeffler's blood serum may be used. We fill the plates to about an eighth of an inch in depth with the mixture and bring the medium rather rapidly to the coagulation point in the autoclave with the door about three-quarters closed. This takes four or five hours. Then the door is completely closed and steam allowed to escape through the valve for about fifteen minutes, after which the valve is closed and the plates are autoclaved for 20 minutes at 15 pounds pressure when they are ready for use. They frequently contain a large amount of water of condensation and this is poured out just before they are inoculated. Bubbles sometimes occur but such plates can be used. Inoculation is made with a platinum loop on the center of the first plate after which a glass spreader is used to distribute the growth on the first plate, using the same spreader to distribute the growth on two succeeding plates. Occasionally 4 plates are used for badly contaminated cultures. As a general rule we are not obliged to replate cultures. Fishings, of course, are made macroscopically to Loeffler's tubes and we find Miss Beckler's suggestion as to the hand lens very helpful.

We ran, as a comparison, duplicate cultures on ascitic agar plates. We found that as far as results went, the media were of practically equal value with perhaps some slight advantage on the side of the Loeffler's blood serum plates in the case of grossly contaminated cultures.

The method is so simple that it is quite possible that other laboratories are using it, though inquiry has not as yet elicited such information. The procedure is one which we can recommend highly to any laboratory making Loeffler's blood serum.

## NOTE ON THE COMMENTS OF REZNIKOFF ON THE USE OF SODIUM SULPHATE AS A PRECIPITANT OF "PSEUDOGLOBULIN"\*

BY PAUL E. HOWE, PRINCETON, N. J.

IN an article on "The Action of Proteins and Blood Serum on Colloidal Gold Solution and Its Quantitative Interpretation,"<sup>1</sup> Reznikoff makes the following statement:

"There is no chemical method for determining proteins which is accurate and practical. Howe's<sup>2</sup> sodium sulphate method was tested against the pure proteins to determine its applicability to these experiments. It was found that neither 14 per cent nor 22.2 per cent sodium sulphate had any effect on the albumin; that the 14 per cent salt precipitated 97.88 per cent of the euglobulin; that the 22.2 per cent salt precipitated 96.4 per cent pseudoglobulin; but that the 14 per cent solution precipitated 89.16 per cent of the pseudoglobulin, thus making the determination of the separate globulins impossible."

In his conclusions, the following occurs:

"10. Howe's method for isolating the globulins was not found to be accurate by testing it with the pure proteins used in these experiments, the euglobulin precipitating agent precipitating 89.16 per cent of the pseudoglobulin."

These statements apply only to the protein preparations used in the experiments presented in the paper. These proteins were prepared by the usual salting out procedures—saturated sodium chloride and half saturated ammonium sulphate. After salting out they "were not dialyzed. . . . but were precipitated with 95 per cent alcohol, centrifuged, washed with 80 per cent alcohol three times, centrifuged each time, and then washed into a dish with 95 per cent alcohol. The mixture was dried by an electric fan and the globulins taken up with 0.1 per cent saline." Furthermore, Dr. Reznikoff states in a personal communication to me that no other globulin precipitant than sodium sulphate was tried on the preparation.

In the absence of suitable controls there is no need for a detailed discussion of the validity of the evidence against sodium sulphate, and incidentally probably all other salts, as a means of separating proteins from blood serum or plasma in general save to say that preparations of pseudoglobulin have been made in this and in another laboratory which gave little or no precipitate with 14.2 per cent sodium sulphate. While Dr. Reznikoff refers only to his particular preparations, the implication in his summary is that his preparations were above reproach and, therefore, the salting out procedure was at fault, which is not the case at all.

\*From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.

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In the last section of the summary the statement that, "New methods for the separation and determination of proteins are necessary before the very obvious and probably important differences of these substances in various clinical conditions can be investigated" is only in part true with regard to both the procedures and the results obtained. The necessity for further work is, however, evident to any one who has worked with proteins. Since the beginning of protein chemistry it has been necessary to resort to salting out, acidification, or dialysis, and in some cases (products of protein hydrolysis) to precipitation with alcohol to obtain various protein "fractions" of blood. Refinements have come especially in choosing a particular salt concentration, acidity, or the dilution of the blood serum or plasma, and in the methods of analysis of the fractions obtained. The advantage of sodium sulphate or other salts over ammonium sulphate, the classical precipitant, lies in the fact that the nitrogen of the protein can be determined directly. There are objections to magnesium sulphate in that, (a), solutions from protein precipitations filter slowly and, to a less extent, (b), a precipitate of magnesium hydroxide is formed when alkali is added in the Kjeldahl procedure. For those who prefer the refractometric procedure of Robertson or the colorimetric procedure of Wu the presence of ammonia is not an important factor. The latter methods of analysis, however, rest fundamentally on "salting out" processes—which the statements of Reznikoff in effect attack. It is not necessary to use sodium sulphate to precipitate the proteins in order to determine the protein nitrogen directly since sodium phosphate, potassium phosphate, or magnesium sulphate will, as we have found, give all of the usual "globulin fractions" as satisfactorily as sodium sulphate. A single precipitation with any salt will, of course, carry with it certain errors, but these are under the circumstances inevitable. We still believe that knowledge with regard to pathologic changes can be greatly enhanced by the use of the existing methods for the determination of proteins.

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CONCERNING THE FORMALDEHYDE-GEL REACTION IN THE  
DIAGNOSIS OF SYPHILIS\*

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BY ROBERT A. KILDUFFE, A.M., M.D., PITTSBURGH, PA.

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**A**MONG the numerous serologic "short-cuts" proposed for the diagnosis of syphilis is the formaldehyde-gel reaction of Gate and Papacostas.<sup>1</sup>

As described the test consists of the addition of two drops of 40 per cent C.P. formaldehyde solution to one c.c. of clear serum, the tube, after mixing, being plugged and incubated for 24 to 48 hours.

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\*From the Laboratories of the Pittsburgh Hospital.  
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A positive reaction is indicated by the formation of a jelly-like clot. In their series of 400 cases an agreement of 85 per cent was claimed with the Wassermann test and the reaction advocated as a simple and reliable means of testing for syphilis in the office of the practitioner.

The only explanation thus far advanced of the mechanism of the reaction is that of Holborrow<sup>2</sup> who believes that the formation of the clot is due to the direct action of the formaldehyde upon the proteins of the serum and who holds it probable that an acid protein has the ability to produce gel formation while alkali protein does not. Gel formation, therefore, he looks upon as an indication of decreased serum alkalinity without indicating its cause. He looks upon the test in the light of his findings with it with much reserve.

Sufferin<sup>3</sup> reports an agreement of 90 per cent with the Wassermann test but his investigations include only eleven cases which at once robs his conclusions of all significance.

Eeker<sup>4</sup> in a series of 500 cases found an agreement with the Wassermann of only 37 per cent and concludes that the reaction is of no diagnostic value "because of its failure to react in clinically and serologically clear-cut cases of syphilis and the occurrence of positive reactions in the absence of the disease."

Similar conclusions were reached by Armangue and Gonzales<sup>5</sup> and by Kingsbury<sup>6</sup> who emphasizes the futility of basing conclusions upon too small a number of cases, such as the series of Sufferin.

Burke<sup>7</sup> in a series of 319 sera obtained an agreement with the Wassermann of 85 per cent but notes the fact that the reaction is prone to give false positive and false negative results and looks upon the test as unsuited for use alone as a means of diagnosis.

In the present communication the results of a comparison of the formaldehyde-gel and Wassermann reactions are reported in a series of 480 sera.

The Wassermann reaction was made after the modification recently described by Kolmer<sup>8</sup> and proposed for standard adoption, experience with this technic having shown it to be remarkably delicate and reliable.<sup>9</sup>

The formaldehyde-gel reaction was made upon the same sera at the same time as the Wassermann.

The sera tested comprised those examined for diagnosis, those from known syphilis and from syphilis under treatment, and a variety of nonsyphilitic conditions from the wards of the Pittsburgh Hospital.

All sera were inactivated for 15 minutes at 56° C. and the majority tested within 24 hours of their receipt, none being over seventy-two hours old.

The technic of the Gate and Papacostas test consisted in the addition of two drops of 40 per cent formalin (Merck) to one c.c. of clear serum in a small tube which was then well shaken and the mixture incubated without plugging the tube in a bacteriologic incubator at 38° C. for 24 hours, after which the reading was made.

The formation of a firm, jelly-like clot was considered a positive reaction. Occasionally a membranous scum or pellicle formed on the surface adherent to the tube at its edges. On jarring the tube, however, this was dislodged show-

ing fluid serum beneath. Such sera were read as negatives. Only positive and negative readings were made, quantitative readings being impossible.

Of the 480 sera tested 80 or 16.6 per cent were Wassermann-positive in varying degree, the remainder being negative.

With the formaldehyde test 170 or 35 per cent were positive; of these only 40 or 8.3 per cent occurred with the Wassermann positive sera, the reaction being entirely negative with 50 per cent of the Wassermann-plus sera.

Of the 400 Wassermann negative sera 130 were positive to the formaldehyde reaction.

It is obvious, therefore, that the formaldehyde-gel reaction will give negative results in syphilis, and positive results in the absence of syphilis in a sufficiently high proportion of cases to render it extremely unreliable as a means of serologic diagnosis and the test should, therefore, not be used for that purpose.

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## A METHOD FOR THE DETERMINATION OF HEMOGLOBIN\*

BY RUSSELL L. HADEN, M.A., M.D., KANSAS CITY, MO.

THE many methods and devices suggested for the estimation of hemoglobin bear witness to the unsatisfactory nature of the determination. The only direct method which is at all practical is the oxygen capacity method of Haldane as adapted by Van Slyke to his blood gas apparatus.<sup>1</sup> This procedure requires practice and technical skill and is too time-consuming for routine use. It should, however, be the method of choice in every case of anemia where a careful and accurate determination of the hemoglobin is of value in diagnosis or prognosis.

All the commonly used clinical methods for the determination of hemoglobin are colorimetric in nature and consequently indirect. The accuracy of the results obtained in all such methods depends upon several factors, principally the standard and the calibration of the instrument. Satisfactory results can be obtained with almost any method or instrument provided certain fundamental considerations be understood and reckoned with.

Probably the most valuable method for routine clinical use is the acid

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hematin method of Sahli. Good results will be obtained if the pipette used is accurately graduated, the comparison tubes are of equal bore, and the standard solution is correctly made. It is unusual, however, to find a Sahli instrument on the open market fulfilling these several requirements. The method is not readily adaptable to the rapid hemoglobin determination on many patients, the tubes cannot be easily transported after the blood specimen has been placed in them, and the readings need to be made at a uniform time after the specimen is obtained.

The Dare method has the advantage of a permanent standard of colored glass and the use of undiluted blood. The instrument is expensive and fragile. It can be used for no other purpose than hemoglobin estimation. Quite widely different readings are obtained with the same blood on different Dare instruments since it is almost impossible to manufacture a colored glass which will give uniform results in colorimetric procedures. It has the added disadvantage that the colors to be matched are shades of red—a color with which it is most difficult to make good colorimetric readings.

The method described below for the determination of hemoglobin has some advantages which make it of value under certain conditions. It was originally devised to supply the need for a method in which the blood specimens could be obtained at the bedside, be sent to the laboratory, and the readings be made when the cell counts were done. The blood is diluted when drawn and requires no further manipulation, the dilution is made in an ordinary red cell counting pipette, it may be easily transported without danger of loss, only a small amount of blood is required, and the determination may be made within any time from five minutes to twenty-four hours after the specimen is obtained. The ease of transportation and the fact that the readings may be made as is most convenient make the method especially valuable wherever it is desirable to obtain the specimen outside and make the determination in a central laboratory as is commonly done in many large hospitals.

The method is an adaptation of the acid hematin method of Sahli to the Hellige colorimeter (universal colorimeter of Autenrieth and Königsberger). The use of this instrument for hemoglobin determination is of course not new, the instrument having been originally devised as a hemoglobinometer and has been used by others for a similar purpose. As ordinarily used, however, a special pipette is necessary and the blood in the unknown and in the standard are in the same dilution. If the blood is diluted 1:100 with the special pipette as is usually done, the color changes over the part of the scale most used are so slight and gradual as to make accurate readings almost impossible. We have found that by so diluting the standard as to make all readings from 30 to 110 per cent fall on the middle third of the wedge of the colorimeter exceedingly sharp color changes are obtained. In the method as given the standard is thus diluted.

In order that readings may be made on the fluid contained in the bulb of small red cell pipettes a thin piece of wood such as a tongue depressor is placed in the bottom of the eup holder. This raises the bottom of the eup on a level with the lower margin of the aperture of the colorimeter through

which the readings are made. It is unusual to find a Hellige colorimeter on which the scale is correctly placed, hence every instrument should be carefully calibrated. The accuracy of the readings will depend in large measure on the care with which the calibration is made. In the preparation of the standard solution we have taken 15.6 grams of hemoglobin per 100 c.c. as equivalent to 100 per cent.<sup>2</sup> The method is described in detail below.

#### CALIBRATION OF THE HELIGE COLORIMETER

With the same solution in both cup and wedge read the colorimeter scale when the color of the solution in the cup matches that in the wedge. Normal blood diluted 1:100 is satisfactory for this purpose. This scale reading determines the 100 per cent mark when the unknown and the standard are equally diluted. Designate this point "Y." Now raise the wedge until its lower end is just above the lower level of the aperture through which the readings are made. This determines the point on the scale equivalent to 0 per cent. This scale reading may be designated "X." The depth of the cup at its bottom is 10 mm. It is evident that the depth of the wedge at the point at which the colors match must be 10 mm.

The percentage of substance in the cup in terms of that in the wedge is:

$$10 \times P \times B = (R-X) \times \frac{10}{Y-X} \times A \times 100$$

Where:

- P is the percentage of substance in the unknown solution
- A is the concentration of the standard
- B is the concentration of the unknown
- R is the reading of the scale at the point at which the colors match
- Y is the point on the scale equivalent to 100 per cent
- X is the point on the scale equivalent to 0 per cent

then

$$P = (R-X) \times \frac{100}{Y-X} \times \frac{A}{B}$$

The unknown blood is diluted 1:100 or is in a concentration of 1 per cent. For simplicity of calculation it is desirable to make the factor  $\frac{100}{Y-X} \times A$  equal a whole number. If this number be 2 the readings will fall on the middle portion of the wedge when the blood is diluted 1:100. In a properly constructed wedge (Y-X) equals 80 mm., hence with the standard in a concentration of 1.6 per cent the calculation will be as follows:

$$P = 2 (R-X)$$

Thus the percentage of hemoglobin in the specimen under consideration can be read off directly by subtracting the zero point from the scale reading and multiplying by two. It is convenient to construct a scale for each instrument showing the hemoglobin in per cent and in grams corresponding to the different scale readings.

#### PREPARATION OF REAGENTS

1. Tenth normal hydrochloric acid. This may be made sufficiently accurate by diluting 11.7 c.c. of concentrated hydrochloric acid with distilled water to a volume of 1.000 c.c.



2. Acid Hematin Standard. Withdraw by venipuncture about 50 c.c. of blood, defibrinate by whipping, and strain through gauze. Determine the hemoglobin content of the defibrinated blood by the oxygen capacity method in the Van Slyke apparatus.<sup>1</sup> Dilute the blood with N/10 hydrochloric acid to make a 20 per cent solution of blood containing 15.6 grams of hemoglobin per 100 c.c. of blood; i.e., if the blood used is found to have a hemoglobin content of 14.0 grams per 100 c.c., then 22 c.c. of the blood must be employed to make a 20 per cent solution of a blood with a hemoglobin content of 15.6 gm. per 100 c.c. Mix well and let stand for 24 hours. Add an equal volume of glycerin. Store in a glass-stoppered bottle, preferably in a cool place away from the light. This solution will keep without fading for months. It represents a 10 per cent solution of a blood containing 15.6 grams of hemoglobin per 100 c.c. From this stock solution any dilution desired, usually 1.6 per cent, may be made. Equal parts of glycerin and N/10 hydrochloric acid are employed as a diluent.

*Procedure.*—Draw up blood from a puncture wound in the ear or finger tip exactly to the 1 mark on a red cell counting pipette. Fill to the 101 mark with N/10 hydrochloric acid. This makes a 1:100 dilution of the blood.

Allow the preparation to stand for 10 minutes or more. At least 10 minutes is required for the conversion of the hemoglobin into acid hematin. There is very little change after this time, so the reading may be made 24 hours or even 48 hours after the dilution is made.

Blow the contents of the pipette into the cup of the colorimeter after discarding the first drop. This is most conveniently done with a small rubber bulb placed over the end of the pipette.

Read the scale at the point at which the color of the unknown matches that of the wedge.

*Calculation.*—If the blood in the standard is so diluted as to make the factor  $\frac{100}{Y-X} \times A$  equal 2, the hemoglobin corresponding to any reading R on the scale is  $2(R-X)$  per cent or  $2(R-X) \times \frac{15.6}{100}$  grams per 100 c.c. of blood.

*Precautions.*—The colorimeter cup must be kept clean by rubbing the inside surfaces with a small moist cotton swab. Even a small amount of acid hematin adhering to the sides will in time make a large error in the readings.

The wedge holding the standard must be shaken daily to keep the acid hematin in suspension. It is best to place the instrument on side when not in use.

The red cell pipette used for the diluting must be accurately graduated. A large percentage of the pipettes on the open market are inaccurate.

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## EDITORIALS

### *Incompatible Blood and Eclampsia*

TO the various hypotheses which have from time to time been offered to explain eclampsia we may now add that of incompatibility between the blood of the mother and that of the fetus. McQuarrie (Bull. Johns Hopkins Hosp., Feb., 1923) in a careful analysis of results, suggests this as a possibility, while emphasizing that the work so far is not conclusive.

Flexner, Pearce and others have observed that agglutinated red blood cells may form hyaline thrombi in the capillaries, thereby inducing focal necrosis in the liver and occasionally also in the kidneys and elsewhere. The reaction in the liver usually is found around the portal vessels. Thrombosis precedes necrosis and is accompanied by intense engorgement. Hemorrhage follows, and typical necrosis develops within twenty-four to forty-eight hours of the injection of hemoagglutinative serum. The process appears to be chiefly mechanical, and the pathologic lesions resemble very much those observed in eclampsia.

Although the blood group, usually, is not determined by the time of birth, McQuarrie has studied a large number of bloods obtained from the umbilical cord and simultaneously from the mother, and finds that of the entire series

24.4 per cent had developed interagglutination at birth. Seventy per cent of all the cases of toxemia were comprised within this 24 per cent who had developed interagglutination. Sixty-four per cent of cases showing interagglutination manifested signs of toxemia, while only 9 per cent of cases without interagglutination showed toxemic symptoms. Of the total number of toxic cases, 93.3 per cent showed incompatibility between the maternal and fetal blood, while only 6.7 per cent of toxic cases showed true compatibility. Toxemia was found to be 16.5 times more likely to occur when the maternal and fetal bloods were incompatible than when they were of the same blood group.

McQuarrie points out that before a definite relationship between agglutination and eclampsia can be assumed it must be shown (1) that the isoagglutination characteristics of the fetus be developed at the time the toxemia appears; (2) that the fetal blood grouping differ from that of the mother; (3) that a break in the placental barrier between the two circulations occur at some point, and (4) that sufficient fetal blood enter the maternal circulation to give rise to the clinical and pathological changes observed.

In a certain number of cases, the blood group has probably been developed within the fetus within the last quarter of pregnancy and this fetal group frequently differs from that of the mother. McQuarrie's work is correlated with the observations of Young, who found that the toxemia of pregnancy is associated with premature separation of the placenta or with placental infarction with subsequent absorption of toxic products. If this be true, it may be possible to hypothecate a break in the placental barrier between the two circulations, and a fetal origin of agglutinated red cells in the maternal liver.

The total volume of blood in the fetus does not exceed 300 to 500 c.c. but it has been pointed out by Ottenberg that a few large clumps of foreign cells cause a more serious reaction than a much larger number of cells in small clumps. McQuarrie estimates that as little as 25 to 50 c.c. of fetal blood might be sufficient to cause severe reaction in the mother.

The author recognizes that his observations, while thoroughly interesting and suggestive, are not conclusive, particularly because there are unexplained exceptions in each of the various classifications of his work, and he outlines further study which may cast additional light upon the subject. The hypothesis is at any rate, attractive.

—W. T. V.

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### *Achlorhydria*

THE greater ease and accuracy of diagnosis with the opaque-meal in gastric pathology as compared with test-meal examination has resulted in a disposition to undervalue information obtainable from gastric analysis. On the contrary, the correlation of roentgen and test-meal findings has enhanced the value of the latter and in certain conditions has enabled us to draw more nearly correct conclusions from gastric analysis. We no longer insist that

achlorhydria must be present before a diagnosis of carcinoma of the stomach is made. No more do we insist upon the presence of hyperchlorhydria in ulcer of the stomach or duodenum. Nevertheless, the findings as made in each case of organic abdominal disease are usually of some value in summing up the diagnostic conclusions.

Increasing interest is developing in conditions associated with achlorhydria, and as more gastric analyses are being performed, achlorhydria is found to be decidedly more prevalent than was thought formerly to be the case. Hurst remarks (*Lancet*, Jan. 20, 1923) that out of 325 unselected cases, achlorhydria was present in 10 per cent. In an additional 9.2 per cent no free acid was found one hour after the test meal, although it was present at other times when the fractional method was employed. The frequent association between achlorhydria and pernicious anemia is well known. Various clinicians have observed that the gastric abnormality is even more constant than is the anemia itself. Achylia has repeatedly been noted, preceding sometimes by years the development of the primary anemia. That achlorhydria in these cases is possibly an hereditary affair is suggested by the finding of achylia in immediate relatives of individuals suffering from pernicious anemia.

In general two schools of thought exist in regard to the cause of this gastric secretory anomaly. There are those who maintain that the disappearance of free hydrochloric acid is secondary to a chronic gastritis. The second school maintains that the absence of acid is primary and that gastritis results only secondarily, from attempts to overwork a constitutionally inferior organ. This is borne out by the observation that in nearly all cases of achlorhydria some acid is present as combined acid, but not in sufficient amounts to become free acid. Furthermore, in such cases, operated upon for some extraneous condition, the gastric mucosa does not appear atrophied and on microscopic examination, the secretory elements are normally present, a phenomenon which does not hold in chronic gastritis. The secretory elements are histologically intact, but functionally inadequate. Finally, under proper treatment, free hydrochloric acid sometimes returns in the achlorhydria of chronic gastritis, such as alcoholic gastritis. This is not true of constitutional achlorhydria. The therapeutic deduction made by Hurst is that individuals suffering from achlorhydria should be furnished with adequate facilities for better digestion so that a chronic gastritis may not develop from constant irritation. Rational treatment consists in the administration of hydrochloric acid, a diet which will not remain too long in the stomach, and thorough mastication of the food.

Hurst lays great emphasis on the relationship between achlorhydria and infection in the mouth and upper respiratory passages. Pus and bacteria entering an anacid stomach are not digested and destroyed in the usual manner, but pass through into the intestines where they may be more readily absorbed and produce further disorder. He is inclined to attribute a certain proportion of appendicitis associated with focal infection to escape past an inefficient barrier rather than absorption directly into the blood stream. He observes that achlorhydria is not infrequently present in chronic arthritis and



that rational treatment of this disease will include local treatment of the stomach to prevent the passage of infectious agents into the intestines. That this is not always the case or that it is not the case in the majority of cases of arthritis, may be shown by the performance of gastric analysis on a series of individuals suffering from this disease.

The value of gastric analysis has increased since the institution of the fractional method of study. The discomfort to the patient and the fact that the results usually are of secondary diagnostic interest, have caused many to dispense with this valuable procedure. Like so many other items of the routine examination, it fails to give, in the majority of instances, any valuable information. But the value of routine examination of any form whatsoever lies in its tendency to bring to light the unsuspected finding in the occasional case.

—W. T. V.

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### *Ambroise Paré\**

THERE is renewed interest in the life and work of the great surgeon of the sixteenth century, Ambroise Paré, and Doctor Packard has given us full information, most attractive in literary form, while the publisher has spared no pains in either text or illustration. Paré is a very striking figure. He was physician to four kings successively. There is still dispute as to whether he was Catholic or Huguenot. Evidently he practiced his profession without regard to the religion of his clientele and, although he lived in the midst of the great Protestant massacre, his historian is unable to say with certainty what his religion was. Possibly he did not have any. We are inclined after reading this charming story, to come to the conclusion that the distinguished surgeon of the sixteenth century was both wise and wily. Of lowly birth he was honored beyond any one else in his profession of the generation in which he lived. Beginning as a barber-surgeon he became the greatest man in the College of Surgeons in France and was able to hold his own even with the dominating, aristocratic, theorizing members of the College of Physicians. Brought up in poverty he became the possessor of many houses in Paris and a comfortable country home; indeed, his wealth enabled him to play the rôle of a philanthropist not only to his poor relations, but to other deserving people. His writings are at the same time punctuated with egotism and modesty. On the same page he tells us of some great cure he wrought where others had failed, and then adds: "I dressed his wounds; God healed them." His record, at least in places, sparkles with wit and elsewhere bares gems of wisdom. Above all, as an author, he is both entertaining and instructive, and this is a combination possessed by but few writers. Running through even the most important of his discoveries there is in the narrative always a personal flavor. When the College of Physicians attacked him, one of the charges was that he was ignorant of

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\*The Life and Times of Ambroise Paré, by Francis R. Packard, M.D., Paul B. Hoeber, New York, 1921.

Latin. To this he pleaded guilty, and one of the things which has made him great is that he had the courage to write in the vernacular. He expressed no shame on his admitting his ignorance of Latin and Greek, but pointed to the fact that he was able to hire translators. Paré made most of his reputation as an army surgeon. He was almost constantly in the field with some besieging army or in some besieged city. Great military leaders strove one with the other to secure his services and he naively tells us that after he had been smuggled into the besieged City of Metz in 1552 the dukes, princes, and other high officials in the city embraced him, supplied him with pipes of wine, and assured him that since he had arrived they would no longer fear grave disaster from the barbs of the enemy; indeed, Paré's "Journey to Metz in 1552" is a most charming story, quite equaled though by several others. This siege was most interesting from many standpoints. It was soon after the introduction of firearms in warfare. In the besieged city were nearly all, or at least a large number, of the heads of the aristocratic families of France, with about 6,000 men, all under the command of the Duke of Guise. On the outside and besieging the city was an army of 120,000 under the Duke of Alva. Guise, wise beyond his generation and apparently acquainted with some of the disasters which had previously befallen beleaguered cities, gave marked attention to the cleanliness of the city, to the storing away of vegetables for the winter, possibly with the thought of scurvy in mind, and to the health of his soldiers and of the citizens. The Duke of Alva swore by his beard that he would capture all these French notables trapped within the walls of Metz and that he would demand for each a princely ransom. Paré was smuggled into the city and, as we have seen, brought with him much cheer and comfort. After sixty-three days the plague having appeared in the besieging army, the Duke of Alva departed with his great hosts, leaving behind many sick and wounded.

It has been stated that Paré was the first to use the ligature to check hemorrhage, especially in amputations. This is not true. Paré himself made no such claim; indeed, when he was attacked for recommending ligatures, he retorted that this method of checking hemorrhage had been recommended by Hippocrates, Galen, Avicenna, John of Vigo, and others. John of Vigo, who preceded Paré about one generation taught that wounds inflicted by firearms are poisonous and recommended that all such wounds should be treated with boiling oil. In his first campaign Paré made the great discovery that this was not necessary. He tells that being compelled to treat a great number of gunshot wounds, following John of Vigo, he applied boiling oil to many, but his supply of oil being exhausted he treated the others by placing on the wounds an emulsion made of the yolks of eggs, oil of roses, and turpentine. "That night I could not sleep at my ease fearing that by lack of cauterization I would find the wounded upon which I had not used the said oil dead from the poison. I raised myself very early to visit them, when beyond my hope I found those to whom I had applied the digestive medicament feeling but little pain, their wounds neither swollen nor inflamed, and having slept through the night. The others to whom I

had applied the boiling oil were feverish, with much pain and swelling about their wounds. Then I determined never again to burn thus so cruelly the poor wounded by arquebuses."

The possibility of infection being disseminated by flies apparently struck Paré. He tells that after the Battle of San Quentin (1557) he visited the battle-field and there found many dead men and horses, with a fearful stench arising from the decomposing bodies. He says that these bodies were covered with a great number of big flies which had procreated themselves from the humidity of the dead bodies. They had tails of green and blue and when disturbed they flew in swarms so great that they made a shadow in the sun. He says: "We heard them buzzing with great wonder; and I believe that there where they settled it would render the air pestilent and cause the plague."

—V. C. V.

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### *The Original Daily Dozen*

CALISTHENIC exercises are enjoying an unprecedented popularity. This may be accounted for, in part at least, by the deed of some good or evil genius who has had the exercises set to music for vietrola accompaniments. That setting up exercises are of ancient evolutionary origin may be suspected by observing the stretchings and yawnings of the family dog or cat after a postprandial nap.

Dingizli has analyzed the Mohammedan prayers and their relationship to general hygiene (Bull. Acad. Med., Paris, Jan. 30, 1923). He suggests that the salaams and obeisances were incorporated in the ritual because their originator recognized the value of periodic exercise of the body. The Mohammedan prayer is at the same time a religious symbol and a cleansing or purification of the body. The true believer makes five prayers daily—the first between dawn and the rising of the sun; the second at noon, when the shadows first commence to grow; the third, before the setting of the sun; the fourth, during twilight, and the fifth at some hour of the night.

The faithful who at each prayer is holding an audience with his Master, is not prepared for such communion until he has rigorously followed certain procedures for the cleansing of the body. The degree of cleansing depends upon the nature of the impurities with which one may have been in contact. The greatest impurity is the accomplishment of the act of procreation. Lesser impurities result from sleep, micturition, defecation, vomiting, loss of blood, etc. The great impurity may be effaced only by bathing the entire body. Even the dirt must be removed from beneath the nails. The lesser impurities may be cleansed by less rigorous methods, such as triple washing of the hands, triple washing of the mouth, cleansing of the teeth, irrigation of the nose, etc. The cleanliness of the water and the locality where the ablutions are performed are also definitely prescribed.

At prayer, the faithful, standing erect, faces the direction of Mecca, directs his hands heavenward and pronounces: *Allah Akbar*, God is great!

He then lowers his arms or crosses them, kneels, recites the first chapter of the Koran and makes a profound reverence, bending forward to the ground. Raising his body he repeats his conviction that God is great and prostrating himself again, he gives himself entirely into the keeping of the Almighty. This is the great moment of prayer. Now the forehead and the nose must touch the ground, a symbol of the nothingness of the man.—dust before the Great Presence. Once again the trunk is elevated, and the legs are crossed beneath the body so that the anterior surfaces of the tibiae and the dorsum of each foot are in contact with the earth. Again the suppliant prostrates himself and recites formulas of glorification. Finally he arises.

This gymnastic procedure must be executed twice in the morning, four times at noon, four times in the afternoon, three times at sunset and four times during the night. There are supplementary genuflections which bring the total to about thirty daily. Cleanliness and suppleness of body are thus developed along with religion in the orthodox Mussulman. Further, the repetition of various prayers exercises the memory as well as the body.

We may assume that Mohammed like Moses was a great hygienist. In all probability neither prophet developed in their entirety the laws attributed to them, but their names stand rather as symbols for the great thinkers of their times. The true believer among the Mohammedans has taken his daily dozen for centuries, and except where infectious disease has decimated his ranks, the results, so they tell us are apparent. Dinguizli remarks on the infrequency of appendicitis in Mussulmans and suggests that it may be due in part at least to the constant exercise of the trunk muscles.

Unfortunately, in Christian and Mohammedan countries alike most of us do not follow even the simple formulas prescribed by the greatest of the ancient hygienists. If we would but follow their teachings the world would be a cleaner and healthier locality.

—W. T. V.

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### *Epidemic Yaws in the Philippines*

IN THE summer of 1922, Surgeon General Ireland appointed a Medical Research Board, of which Colonel George R. Callender is chairman, and sent it to Manila. The United States has a right to be proud of the scientific and medical work done in the Philippines since our occupation. Certain army medical officers have seized the opportunities presented in this new tropical field and have made most valuable contributions to our knowledge of the bacteriology and epidemiology of certain diseases there prevalent. Under the guidance of Dr. Paul Freer, the Bureau of Science in the Philippines came to be one of the most important world centers for the study of tropical diseases. Under more recent administrations this institute has apparently been allowed to lapse into desuetude. We hope it will be revived, but if this does not occur we are sure that the Medical Research Board of the Army, referred to above, will make valuable contributions to medical science.



Under date of October 30, 1922, Colonel Callender informs us that there is only four miles out of Manila an epidemic focus of yaws, with about 1,000 cases within an area of approximately four square miles or less. The Board is now able to obtain arsphenamine at the low price of 25c per tube, and within a short time we will undoubtedly have more decisive evidence concerning the curative properties of this preparation in the treatment of yaws.

The Board, in conjunction with Dr. Albert, is also studying infantile beriberi, which has been a large factor in the infantile death rate in Manila. We congratulate the Surgeon General on his wisdom in appointing the Medical Research Board for the Philippines and for selecting as its director Colonel Callender. We may rest assured that no opportunity to add to our knowledge of the diseases prevalent in the Philippines will escape this Commission.

—V. C. V.

## BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, St. Elizabeth's Hospital, Richmond, Va.)

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### *Hawk's Physiological Chemistry\**

THE eighth edition of any book requires no introduction. Because of the rapid development of new methods in the field of physiologic chemistry, a book on this subject which is not frequently revised becomes out of date in a remarkably short time. The eighth edition of *Practical Physiological Chemistry* receives the same general treatment that has characterized previous editions. The chemistry and biochemistry of enzymes, of food elements, of secretions, and the various body tissues are discussed in detail. Illustrative laboratory tests are included under each subject together with discussion of the practical application of the known facts to clinical laboratory study. This book accomplishes what few others have succeeded in doing; it is at the same time a laboratory guide for the undergraduate student and an excellent reference book for the experienced worker.

The newer practical methods of analysis are incorporated and brought up-to-date. This is seen particularly in the chapters on blood analysis and gastric analysis. A division devoted to respiration and acidosis contains highly instructive information. In the chapter on metabolism, considerable attention is devoted to the biochemistry of deficiency diseases. Two new tests are included in an addendum. These are quantitative tests for sugar and albumin in the urine, as devised by Benedict, Folin and Hawk, for routine insurance work.

The book deserves the popularity which has been accorded it in the past.

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### *Books Received*

THE MICROSCOPICAL EXAMINATION OF FOODS AND DRUGS. By Henry G. Greenish, F.I.C., F. L. S. Professor of Pharmaceutics to the Pharmaceutical Society of Great Britain and in the University of London; Director of the Pharmacy Research Laboratory; Docteur (H. C.) de l'Université de Paris; Hanbury Gold Medallist, 1917; Joint Editor of the *British Pharmacopeia*, 1914; Late Member of the Board of Examiners of the Pharmaceutical Society of Great Britain; Late External Examiner in *Materia Medica* and Pharmacy to the University of Birmingham. A Practical Introduction to the Methods adopted in the Microscopical examination of Foods and Drugs, in the entire, crushed and powdered states. Third edition. Cloth. Price \$4.50. Pp. 386 with 209 illustrations. Philadelphia: P. Blakiston's Son & Co., 1012 Walnut Street. 1923.

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\**Practical Physiological Chemistry*. By Philip E. Hawk, M.S., Ph.D., Professor of Physiological Chemistry and Toxicology in the Jefferson Medical College of Philadelphia. With two full-page plates of absorption spectra in colors. Four additional full-page color plates and one hundred and ninety-seven figures of which twelve are in colors. A book designed for use in courses of *Practical Physiological Chemistry* in Schools of Medicine and of Science. Eighth edition, revised. Cloth. Price \$5.00. Pp. 693. Philadelphia: P. Blakiston's Son & Co., 1012 Walnut Street. 1923.

A MANUAL OF PHARMACOLOGY and its applications to Therapeutics and Toxicology. By Torald Sollmann, M.D. Professor of Pharmacology and Materia Medica in the School of Medicine of Western Reserve University, Cleveland. Second Edition, entirely reset. Cloth. Price \$5.50. Pp. 1006. Philadelphia and London: W. B. Saunders Company. 1922.

GENERAL BACTERIOLOGY. A textbook by Edwin O. Jordan, Ph.D., Professor of Bacteriology in the University of Chicago and in the Rush Medical College. Seventh Edition. Thoroughly revised. Cloth. Price \$5.00. Pp. 744. Fully illustrated. Philadelphia and London: W. B. Saunders Company. 1922.

CLINICAL MEDICINE. I. TUESDAY CLINICS AT THE JOHNS HOPKINS HOSPITAL. By Lewellys F. Barker, M.D., LL.D., Professor of Medicine, Emeritus, Johns Hopkins University; Visiting Physician to Johns Hopkins Hospital, Baltimore. Cloth. Price \$7.00. Pp. 617. Illustrated. Philadelphia and London: W. B. Saunders Company. 1922.

THE CHEMISTRY OF TUBERCULOSIS. By H. Gideon Wells, M.D., Ph.D., Director of the Otho S. A. Sprague Memorial Institute, Professor of Pathology in the University of Chicago and in Rush Medical College; Lydia M. DeWitt, M.D., A.M., Member of the Otho S. A. Sprague Memorial Institute, Associate Professor of Pathology in the University of Chicago and in Rush Medical College; Esmond R. Long, Ph.D., Assistant Professor of Pathology in the University of Chicago and in Rush Medical College. Cloth, price \$5.00. Pp. 438. Baltimore, Williams and Wilkins Company.

# *The Journal of Laboratory and Clinical Medicine*

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## *ORIGINAL ARTICLES*

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### WHITE MICE AND THE ASSAY OF INSULIN\*

#### PRELIMINARY REPORT

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BY DONALD T. FRASER, TORONTO, CAN.

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THE physiologic assay of insulin presents problems of considerable complexity and at the same time problems of moment, since they involve both the sale and the clinical use of the product. It is highly desirable in regard to the latter for the clinician to have assurance that the dose he wishes to administer to the patient may be gauged from the potency stamped on the vial. The manufacturer, on the other hand must have confidence that such potency bears a close approximation to accuracy in that the extract must be sold on the basis of "units."

It is very apparent that in an attempt to reduce the factor of the individual physiologic variation of the test animal to a minimum one must multiply the numbers of such animals very considerably. In the case of rabbits such a measure involves a very material increase in expenditure of time and adds to the cost of production. The unit of insulin is essentially  $\frac{1}{5}$  of the amount in c.c. required within four hours to lower the normal blood sugar of a rabbit calculated on the basis of 2 Kg., to a point approximating 0.045 per cent at which point the animal usually manifests convulsions.<sup>1</sup> The correlation of this unit with "carbohydrate equivalent" as determined by clinical observation on carefully selected and controlled diabetic patients will require time. Indeed, one may perhaps not expect more than an approximation and not an exact correlation since the individual patients on whom the assay is being conducted may manifest a change in carbohydrate toler-

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\*From the Connaught Antitoxin Laboratories, University of Toronto, Toronto, Ontario.  
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ance from time to time. In addition, a further complication factor is the occasional fall in potency of the extract owing to certain unknown factors causing deterioration. The use of depancreatized dogs for purposes of assay presents many difficulties and requires constant administration of relatively large amounts of extract.

In July, 1922, some experiments were carried out on mice in the hope that by their use a more rapid and less expensive method of assay might be evolved. At that time the extract was not available for extensive use, and the work was not resumed until October. It was found that mice were susceptible in a high degree to insulin. In the early experiments the extract was given intravenously. After considerable experimentation it was established that intraperitoneal injection was more rapid in its effects and acted in higher dilutions than by the intravenous route. Consequently the latter method was discontinued. Some inconsistent results obtained led one to suspect that the Ph. of the saline used in making the dilutions was a factor to be taken into consideration. Consequently, the saline used subsequently for all dilutions was buffered after the manner recommended by Alice C. Evans<sup>2</sup> and a Ph. value of 6.8 was adopted.

The white mice used were obtained from local dealers with no guarantee as to purity of stock. The average weight was about 18 gms., and when possible, such a weight was chosen for use. As a routine procedure it was found advisable to use starved mice. Mice starved for 24 hours and immediately given oats after injection manifest a very marked tolerance to the effect of insulin. The tolerance under these conditions is such that a mouse may withstand, without any evidence of reaction, a dose of one hundred times the amount which will produce convulsions in a mouse which has been starved 24 hours and which has not been fed during the period of the test. Unstarved mice have not shown as consistent results as starved. As a rule they are less sensitive. The length of starvation, after a good deal of experimentation, was chosen as 24 hours. It is necessary to inspect the test animals closely to determine whether or not some of the mice show signs of weakness. Those that are not normal are rejected as unsuitable for testing purposes. The diet previous to starvation consisted in a plentiful supply of carrots and oats. A period of a few days was allowed to elapse before the mice were used for a subsequent test. No evidence was found to show that repeated use of the same mice was inadvisable. No consistent relationship between the weight of the mice and the time of onset of convulsions was established.

The amount of the injected fluid was kept constant, namely, 0.25 c.c. A 26-gauge needle and 0.5 c.c. syringe calibrated in  $\frac{1}{20}$  were used. Standard glassware was used throughout. Dilutions were made so that the following amounts (in c.c.) of insulin were contained in 0.25 c.c. buffered saline Ph. 6.8: 0.01, 0.0075, 0.005, 0.00375, 0.0025, 0.0015, 0.00075, 0.0005, 0.00025. Lower dilutions were occasionally used. All glassware and saline were sterile.

On intraperitoneal injection of insulin there is a latent period of about 20 minutes or longer before symptoms become manifest. During this period the activity of the mice is sometimes decreased but frequently remains nor-

mal. Those mice which receive the larger injections of insulin become less active and finally motionless. At the end of the latent period they frequently exhibit postural eccentricities, clinging to the top or the side of the wire cage, or with the nose pressed firmly through its mesh. Respiration is increased in rate and excursion. Two types of reaction ensue. In one type ataxia and paresis predominate, in the other, convulsions. At present these types of reaction are both considered characteristic. The ataxia is first manifested in the gait of the mouse. The back is somewhat arched, the belly held further from the table than normal, and the mouse moves with a waddling motion. The posterior limbs rapidly become paretic and are extended posteriorly and laterally. The condition may proceed to almost complete paralysis. Before the latter stage convulsions sometimes ensue either spontaneously or in response to a sudden stimulus. The second type of reaction is as a rule of sudden onset and frequently occurs spontaneously. One may, however, often anticipate its spontaneity and induce a seizure by a sudden stimulus. Dropping the mouse from a height of a few inches will induce convulsions in an animal which would otherwise probably not evidence a seizure for some five or ten minutes. The convulsions are clonic and as a rule violent. The head is markedly retracted. The seizure may last 20 seconds or longer and recur if dextrose is not given. To be regarded as a typical reaction in either type the animal must recover within five minutes after the intraperitoneal injection of 0.25 c.c. of 15 per cent dextrose in 0.9 per cent saline. If, however, the mouse is allowed to remain ataxic too long, recovery may not occur, or be long delayed. The period of observation after injection of the extract has been somewhat arbitrarily set at two hours. Three mice at least should be used for each dilution. The highest dilution at which a typical reaction is evidenced is regarded as the end point. The expression of this end point has been designated as the minimum effective dose (M.E.D.).

Owing to the somewhat unsatisfactory methods of assay on rabbits and the difficulty of correlating the carbohydrate equivalent obtained in the clinics, it has been found impossible to express definitely the results obtained with mice in terms of units. It would appear, however, that 0.0025 c.c. of insulin is approximately equivalent to 5 rabbit units per c.c., which in turn is the equivalent of between 1-1½ grams of carbohydrate as determined on patients with severe diabetes.<sup>3</sup> The most potent extract tested up to the present has been one that produced convulsions with 0.0005 c.c. of extract. On the basis of 0.0025 c.c. insulin being the equivalent with mice of 5 units when rabbits are used for assay one may calculate the potency of an extract from the M.E.D. Thus a M.E.D. of 0.0005 represents a potency of 25 units per c.c.; a M.E.D. of 0.0015 represents 10 units per c.c.

It sometimes happens that a mouse which has received a higher dilution, that is, less extract, will evidence convulsions before one which has received a lower dilution. This is, however, not the rule. Occasionally a mouse seems to be tolerant. By the use of three or more mice for each dilution this variability may be eliminated.

The method lends itself admirably to the testing of unconcentrated ex-

tracts. If 0.0025 c.c. approximates 5 units per c.c. and one obtains a typical reaction with say 1 c.c. of dilute extract, which amount may be safely injected without danger, one may assume that the potency is somewhat in the neighborhood of 5 units per 500 c.c. It has also been found a useful method in laboratory investigations where the amount of available material for assay is small.

#### CONCLUSIONS

1. Starved mice are very susceptible to the intraperitoneal injection of insulin.
2. Mice fed immediately after the injection of insulin are very tolerant to its effect.
3. The correlation of the unit as determined by the use of rabbits with the unit as determined by the use of mice has been attempted; 0.0025 c.c. is approximately the equivalent of 5 (rabbit) units per c.c.

#### REFERENCES

- <sup>1</sup>The expression of the unit has been modified since the original publication appearing in the *Am. Jour. Physiology*, lxii, No. 1.
- <sup>2</sup>*Jour. of Infect. Dis.*, 1922, xxx, 95.
- <sup>3</sup>Personal communication, Graham, Campbell, Fletcher, Toronto General Hospital, Diabetic Clinic.

# CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

BY A. LEVINSON, M.D., CHICAGO, ILL.

*(Continued from page 369.)*

## BLOOD

### INDICATIONS FOR EXAMINATION OF BLOOD

IN every disease of infancy and childhood no matter what the diagnosis, the patient's blood should be examined. In many cases, the examination may be limited to the cell elements and hemoglobin for which only a few drops of blood are required.

If congenital or acquired syphilis is suspected, a Wassermann test should be done on the blood. For this purpose, 3 to 5 c.c. of blood should be removed from the patient.

If diabetes is suspected, the blood sugar should be determined. The amount of blood required for this examination depends on the chemical method employed. For the Lewis-Benedict or the Folin-Wu method, 2 c.c. is required; for the Kowarsky method, 0.5 c.c. and for the Epstein method 0.2 c.c. suffice.

If nitrogen retention is suspected, the blood should be examined for the amount of nonprotein nitrogen for which 2 c.c. of blood is required. Some consider the uric acid determination as even more important than the nonprotein nitrogen. The amount of blood required varies between 2 to 4 c.c. depending on the method used. Urea nitrogen also requires 2 c.c. of blood. To throw light on the prognosis of nephritis creatinine should be determined. This examination requires 3 c.c. of blood. All constituents of nonprotein nitrogen of clinical importance may, however, be examined by taking only 5 c.c. of blood.

When acidosis is suspected, as in severe cases of diabetes, diarrhea or infections, the blood should be examined for the alkali reserve or its  $\text{CO}_2$  combining power for which 0.5 to 1 c.c. of blood is required.

In rickets and tetany the calcium content of the blood may be examined; 3 to 5 c.c. being required for the examination.

Of some clinical importance is also the determination of cholesterol in diabetes for which examination 3 c.c. of blood is required and the determination of chlorides in nephritis with threatening edema for which examination 0.1 to 0.2 c.c. of blood is required.

### METHODS OF OBTAINING BLOOD

When only a few drops of blood are required, as for the determination of the cell elements, hemoglobin and coagulation-time, the blood can be obtained by piercing the lobe of the ear or the finger with a sharp scalpel or



needle. When larger quantities of blood are required such as for the Wassermann test and blood chemistry, the blood may be obtained by making a large incision with scalpel in the patient's heel or by making a long incision in the intrascapular region and applying an old fashioned dry cup for withdrawing the blood. Both of these methods, although practiced by some physicians are, however, time-consuming and are unsatisfactory, especially for blood chemistry. In such cases, it is best to withdraw blood from some vein in the body.

In older children it may be possible to remove blood for examination from a vein on the arm or at the ankle. The technic is the same as that in adults which is as follows: The bend of the elbow or the internal surface of the ankle is selected as the site of puncture. A tourniquet (a soft rubber tube, a wide strip of gauze or even a handkerchief), is applied around the

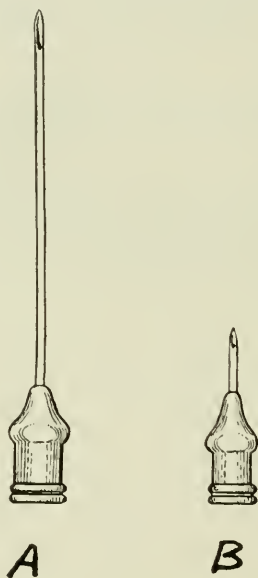


Fig. 10.—Needles employed for removal of blood from median or external jugular veins (*A*) and from the longitudinal sinus (*B*). Needles are shown in their actual size.

limb, 1 to 1½ inches above the point of puncture. The tourniquet should be tight enough to obstruct the venous, but not the arterial circulation. The limb is held by an assistant or is rested on the edge of a table and the area of operation is washed with alcohol and iodine. A 16- to 18-gauge needle, 1 to 1½ inches in length (Fig. 10*A*), fastened to a dry Record syringe is now introduced under the skin overlying the vein and pushed forward into the vein. The skin over the vein is preferably rolled between the thumb and index finger of the operator's left hand to facilitate the introduction of the needle into the vein. As soon as blood appears in the syringe, the plunger of the syringe should be withdrawn carefully until the desired amount of blood is obtained (Fig. 11). The tourniquet is now removed and a gauze bandage is applied around the limb over the area of puncture. No collodion is necessary.

In infants and young children where the veins are too small or where no veins can be distinguished on the skin, blood may be withdrawn from the jugular vein or from the longitudinal sinus.

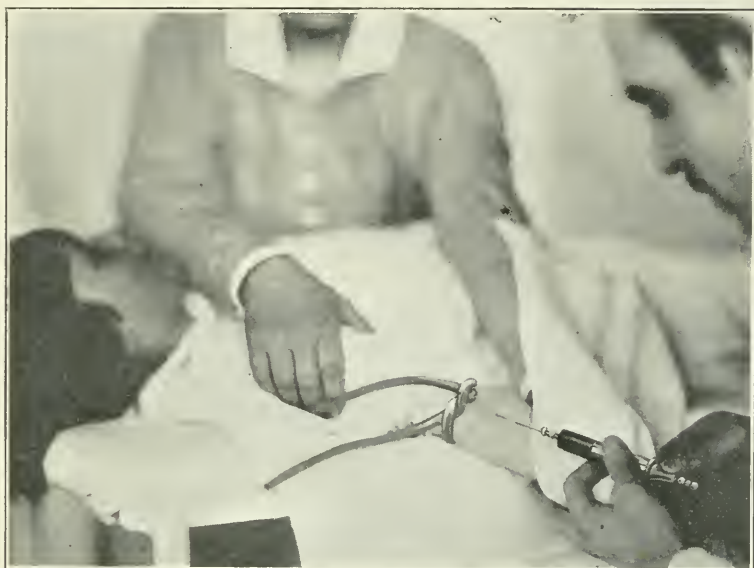


Fig. 11.—Removal of blood from median vein.



Fig. 12.—Removal of blood from external jugular vein.

**JUGULAR VEIN PUNCTURE.**—The child's arms are held closely to the trunk by a tightly wrapped sheet or blanket, the upper edge of which comes only to the shoulders. The child is placed on its back on a table with a small pillow or pad under the shoulders, allowing the head to fall slightly backwards. The chin is turned to the shoulder thus bringing the jugular vein into prominence and into the most accessible position. It may be made to stand out more prominently by the application of pressure just above the clavicle. The skin is cleaned with iodine and alcohol. A 16- to 18-gauge needle, 1 to  $1\frac{1}{2}$  inches in length, attached to a 10 c.c. syringe is introduced into the outstanding vein. The needle is introduced from above downward in a line with the vein (Fig. 12). It is best to pierce the needle through the skin only by the first thrust and then slowly introduce the needle into the vein.



Fig. 13.—Removal of blood from longitudinal sinus.

**LONGITUDINAL SINUS PUNCTURE.**—The child is placed in the recumbent posture with the head at the edge of table. It is best to envelop a sheet around child to prevent struggling. The hair over the anterior fontanelle is shaven and the scalp over this area is washed with alcohol and ether. A 16- to 18-gauge needle,  $\frac{1}{4}$  to  $\frac{1}{2}$  inch in length (Fig. 10B), attached to a Luer syringe is now introduced at the lowermost portion of the posterior angle of the fontanelle, the needle pointing slightly backwards for a distance of  $\frac{1}{4}$  to  $\frac{3}{8}$  of a cm. When the longitudinal sinus is reached, dark red blood appears in the syringe (Fig. 13). The desired amount of blood is now slowly withdrawn and collodion is applied to the puncture. If blood continues to ooze from the opening, the patient's head should be raised on a higher level than the rest of the body. If even this does not stop the bleeding, a tight bandage should be applied around the head.

**PREVENTION OF CLOTTING.**—For the determination of the cell elements, hemoglobin and Wassermann test, no precaution has to be taken to prevent the blood from clotting. For the chemical analysis of the blood it is important that the blood remain unclotted. For this purpose sodium citrate, potassium oxalate or lithium oxalate should be used, the two latter being preferable. In using potassium oxalate, 0.4 c.c. of a 2 per cent solution is employed for 5 c.c. of blood and 0.8 c.c. of a 2 per cent solution for 10 c.c. of blood. The oxalate solution is allowed to dry in the oven at the bottom of the bottle in which the blood is to be collected. A very simple way of preventing clotting of the blood is to run the oxalate solution through the needle and syringe before taking the blood. Even more simple is the usage of 2 to 3 crystals of potassium oxalate in the test tube in which the blood is to be collected. Lithium oxalate, which is considered the best anticoagulant, can be prepared only where good laboratory facilities are available.

**CONTAINERS.**—The blood should be collected directly from the needle in the vein or from the syringe into a wide-mouthed specimen bottle, 10 to 15 c.c. capacity. The receptacle should contain no water, for water tends to hemolyze the blood, a condition that affects the chemical reaction and makes for inaccuracies in the chemical reading. Constant shaking of the bottle is necessary while the blood is being collected so that the blood may be thoroughly mixed with the anticoagulant.

**TIME ELEMENT.**—For chemical examination blood should be withdrawn from the patient before breakfast, as food has a tendency to increase the concentration in the blood of the chemical substance to be examined. It has been found, for instance, that blood sugar increases right after the ingestion of food and that the increase continues from one to two hours. The same is true to a lesser degree of urea. The blood should be examined as soon as possible after it has been removed from the body, at least within twelve hours.

TABLE I  
REMOVAL OF BLOOD FOR DIAGNOSTIC PURPOSES

DETERMINATION	AMOUNT NECESSARY	METHOD OF REMOVAL	
Cell count	Few drops	Ear or toe by sharp scalpel	
Differential count			
Coagulation	1 to 2 drops	“ “	
Wassermann test	3 to 5 c.c.	Cut in heel Needle in median vein (older children) Needle in jugular vein or longitudinal sinus (in infants)	
Blood chemistry			
Nonprotein nitrogen	2 c.c.	} 5 c.c. for determination of nonprotein nitrogen and its constituents	Same as for Wassermann except that an anticoagulant must be added
Urea nitrogen	2 c.c.		
Creatinine	3 c.c.		
Uric acid	2 c.c.		
Sugar	0.2 to 2 c.c.		
Cholesterol	3 c.c.		
Chlorides	0.2 c.c.		
Alkali reserve	0.5 to 1 c.c.		Cut in heel or needle in vein
Blood culture	1 to 3 c.c.		Needle in vein
Blood matching	2 drops to 1 c.c.		Heel or vein
Fragility	few drops to 2 c.c.		Heel or vein



## TECHNIC OF CELL COUNTING.—

1. *Red Cell Count*.—After piercing the ear or finger with a sterilized needle or scalpel, blood is drawn up in the red blood cell pipette (Thomas Zeiss 1-101) to mark 0.5 and Hayem's solution (Mercuric Chloride 0.5 gm.; sodium sulphate 5.0 gm.; sodium chloride 2.0 gm.; distilled water 200 c.c.) to mark 101. The pipette is shaken and a drop is placed in the counting chamber. The cells contained in 100 small squares are counted and the number obtained is multiplied by 8000, or the cells in 80 squares are counted and the result multiplied by 10,000 by adding four zeros to the number obtained. This gives the red cell content per cm.

2. *White Cell Count*.—Blood is drawn up in the white cell counting chamber (1-11) to mark 0.5 and acetic acid (1.5 per cent) to mark 11. The cells in several squares are counted, the average for one square obtained and this number is multiplied by 200, to get the content of white cells per cm.

3. *Differential Cell Count*.—A drop of blood is spread on a slide with the edge of a cover-glass or of another slide or with cigarette paper. After the blood has dried in the air, the smear is covered with Wright stain for one minute followed by the addition of distilled water, drop by drop, for 4 or 5 minutes (depending on the strength of the Wright stain). The slide is then washed off with plain water, blotted and examined. At least 100 white cells should be counted and recorded. The red cells should have been noted for their shape, presence or absence of nuclei and for abnormal cells. The white cells should be counted according to their types (small and large lymphocytes, transitional, neutrophiles, eosinophiles and basophiles). Bacteria and parasites should also be noted.

INTERPRETATION OF CELL ELEMENTS IN BLOOD.—There are several important points to be kept in mind in connection with the determination of the cell elements in the blood of children.

TABLE II

EXAMPLE OF THE DISTRIBUTION OF THE CELL ELEMENTS IN THE BLOOD OF INFANTS AND CHILDREN.

AGE	HR.	RED	WHITE	N	SM.	LM.	T	E
3 days	95	7,500,000	17,200	49	34	15	12	
7 weeks	90	3,980,000	8,200	14	77	5	3	1
next day	90	4,100,000	9,800	21	70	4	5	
next day	80	4,000,000	9,860	11	81	3	3	2
3 months	85	4,300,000	10,000	12	67	12	5	1
6 months	80	4,400,000	12,200	22	63	13	2	0
8 months	75	4,200,000	11,500	37	41	18	3	1
3 years	90	4,600,000	13,200	46	38	4	6	6

The number of red blood cells is high in the newborn, usually reaching over 6,000,000 per cm. and is low during infancy and childhood averaging 4,000,000 per cm. No diagnosis of anemia should therefore be made in infants and children unless the red cells are markedly below 4,000,000 per cm. In newly born babies, especially those born prematurely, or those showing marked jaundice, nucleated red blood cells are commonly found. Later in

infancy and childhood, however, nucleated red cells indicate a pathologic condition.

The leucocytes in the newborn vary between 15,000 to 20,000 per cm., 60 to 70 per cent of the number being polymorphonuclear in type. From the second week on, extending all through infancy and childhood, the number of leucocytes varies between 10,000 and 15,000, with a preponderance of lymphocytes, the number of lymphocytes varying between 40 to 60 per cent. The eosinophiles vary between 1 and 3 per cent. Transitional cells range between 2 and 6 per cent except in the newborn when their number may be still higher (Table II.) The blood platelets vary between 250,000 to 300,000 per cm. all through infancy and childhood.

With these normal variations in mind, the structural examination of the blood in children assumes greater significance. A leucocytosis by itself means very little if anything, unless it is very high, such as 20,000 to 25,000. A leucocytosis with a relative neutrophilia indicates infection. Leucopenia is important because of its presence in measles, german measles, chicken pox, influenza and typhoid (Table III). It must be understood, however, that the leucopenia is present only in certain stages of these diseases, usually in the early stages, the blood changing to a leucocytosis toward the end of the disease or when complications take place.

TABLE III  
LEUCOCYTIC CHANGES IN ACUTE INFECTIOUS DISEASES IN CHILDREN

Scarlet	Marked leucocytosis and neutrophilia, beginning with the prodromal symptoms, rising still higher on appearance of rash and gradually declining with improvement of the patient. Lymphocytes are diminished, increasing gradually with improvement. Eosinophiles are high during eruptive stage, at times reaching to 25 per cent.
Measles	Leucopenia and neutrophilia returning to normal when rash is fully developed.
German Measles	Leucopenia and relative lymphocytosis.
Chicken Pox	Leucopenia and leucocytosis.
Diphtheria	Leucocytosis and neutrophilia. Eosinophiles diminished or absent.
Erysipelas	Leucocytosis and neutrophilia in proportion to severity. Eosinophiles diminished or absent.
Mumps	Moderate leucocytosis, predominance of lymphocytes.
Grippe	Leucocytosis and neutrophilia.
Epidemic Influenza	Leucopenia and lymphocytosis early. Neutrophilic leucocytosis lasting several days on recovery or on onset of complication.
Pertussis	Leucocytosis and lymphocytosis.
Pneumonia	Marked leucocytosis and neutrophilia. Leucocytes ranging between 20,000 to 80,000; neutrophiles ranging between 70 to 98 per cent. Eosinophiles absent throughout disease but reappear on day of crisis.
Meningococcus Meningitis	Leucocytosis and neutrophilia.
Typhoid	Leucopenia and lymphocytosis. Eosinophiles diminished or absent. There is often a leucocytosis on the first day of the disease.

An eosinophilia over 4 per cent is present in eczema, asthma or intestinal parasites, reaching in some cases as high as 20 to 30 per cent. On the other hand, eosinophiles are absent in certain infectious diseases (Table III).

Arnett index and Opsonic index, at one time quite popular are not commonly employed now as a clinical aid. The same is true with inclusion bodies at one time considered specific for scarlet fever.

**HEMOGLOBIN.**—Hemoglobin is most easily determined by the Talquist hemoglobinometer, an instrument sufficiently accurate for clinical purposes.

At birth, the hemoglobin is usually over 100 per cent compared to the adult standard. During infancy, however, the hemoglobin is usually below that of the normal adult. During the school age, most children present a marked anemia spoken of in the literature as "school anemia." This possibly results from the overexertion and fatigue to which many modern school children are subjected. No diagnosis of organic anemia or disease of the blood should be made on the mere lowering of hemoglobin. One must take into consideration the number and type of cells, the hemoglobin and the color index (Table IV).

TABLE IV  
BLOOD FINDINGS IN ANEMIAS OF INFANTS AND CHILDREN

TYPE	RED CELLS	HB.	COLOR INDEX	WHITE CELLS	TYPE OF WHITE BLOOD CELLS	ABNORMAL CELLS
Secondary anemia	Diminished according to degree of anemia	Diminished	1 or below	Slightly increased	Lymphocytes relatively increased	Poikilocytosis
Von Jaksch anemia	Diminished; 2,000,000 or less	Diminished to 20-25%	Normal or below	Increased; 25,000 to 60,000	Mono- or polymorphonuclear Eosinophiles increased	Nucleated red; mainly normoblasts; in severe cases megaloblasts
Acute lymphatic leukemia	Diminished; 2,000,000 to 1,000,000	20 to 30%	1 or below	Greatly increased; 50,000-100,000	Lymphocytosis 90-98%; chiefly large lymphocytes	
Chronic lymphatic leukemia	Diminished; 2,000,000 to 1,000,000		1 or below		Lymphocytosis; chiefly small lymphocytes	
Spleno-myelogenous leukemia	Diminished; 2,000,000 to 1,000,000	Diminished; 20 to 30%	1 or below	50,000 to 200,000	Neutrophiles relatively increased Eosinophiles increased	Myelocytes and mast cells in large numbers
Chlorosis	Diminished; 3,500,000 to 2,500,000	35 to 40%	1 or below	Normal	Lymphocytes relatively increased	Poikilocytosis
Pernicious anemia	Greatly diminished. May fall to less than 1,000,000	Diminished as low as 20%	Above 1	Markedly diminished	Lymphocytes relatively increased	Nucleated red; more megaloblasts than normoblasts; Poikilocytosis

**COAGULATION.**—Coagulation of blood is both an interesting and important phenomenon. It is interesting to see a body tissue that is fluid while in the body coagulate as soon as it leaves the body. Normally blood coagu-

lates in 5 to 10 minutes after it leaves the body. Jaundice, sepsis, syphilis and hemophilia retard the coagulation of blood. In the first three diseases, the delay of coagulation is due to the hemolytic processes going on in the blood.

The following table from Ottenberg shows the importance of coagulation in differential diagnosis (Table V). Of utmost importance is the determination of coagulation time in surgery. No operation should be performed on a child without previous determination of the coagulation time of the patient's blood.

TABLE V

COAGULATION AS AN AID IN DIFFERENTIAL DIAGNOSIS OF HEMORRHAGIC DISEASES IN CHILDREN

	COAGULATION	BLEEDING TIME	PLATELETS
Hemophilia .....	Greatly prolonged	Normal or plus	Normal
Hemorrhage of newborn .....	Greatly prolonged	Prolonged	
Purpura hemorrhagica .....	Normal	Greatly prolonged	Greatly decreased
Purpura, secondary to			
1. Jaundice .....	Prolonged		
2. Chloroform .....	Prolonged		
3. Scurvy .....	Normal	Normal	Normal
Purpura simplex } .....	Normal	Normal	Normal
Arthritic purpura } .....			
Visceral purpura } .....			

The mechanism of hemophilia is still unknown. It has been found, however, that there are two types of hemophilia; one due to calcipriva, and another due to thrombopriva. In the first type of cases, the calcium in the blood is diminished, and in the other type, the calcium in the blood is normal, but the amount of thrombin is lessened.

Therapeutically, it is often desirable to prevent coagulation of the blood. This is most important in cases where large quantities of blood have to be transfused into a child. Two-tenths per cent sodium citrate has been found useful in preventing coagulation.

**BLOOD CULTURE.**—A positive blood culture is of great assistance in diagnosis as it ascertains the causative organism, providing it is known that the culture media was not contaminated. A negative blood culture does not exclude the presence of bacterial organisms in the blood. Blood culture is usually most positive early in the disease. This is especially true in typhoid and pneumonia. It is found to be most positive when the patient has a high temperature.

**Technic.**—Three to 5 c.c. of blood is withdrawn from the median or jugular vein or from the longitudinal sinus, by means of a syringe (method described above). The blood is introduced into a flask containing 100 or 200 c.c. of sterile glucose broth. This is incubated for 24 hours and examined for the respective bacteria. The culture should be returned to the incubator and examined at intervals of 24 hours for a period of one week. This should be done in order to detect the more slowly growing organisms occasionally encountered, such as a poorly growing streptococcus viridans.



**AGGLUTINATION.**—When blood serum agglutinates a certain bacterial organism, it may be taken as evidence that the body is invaded by that particular organism.

Agglutination is mainly used in the diagnosis of typhoid, paratyphoid and dysentery. The agglutination test for typhoid is known as the Widal test. The technic is as follows:

A drop of blood serum or dried blood is diluted with 24 drops of normal salt solution. A drop of suspension of typhoid bacilli, which may be obtained from commercial laboratories, is now added to the diluted blood and examined on a hang-drop slide or on a flat slide. If the patient suffers from typhoid fever, the typhoid bacilli clump and if living bacilli are used, they become motionless in a few minutes.

The reaction may be obtained macroscopically by adding typhoid organisms to diluted blood serum and allowing to incubate overnight.

The Widal test is usually negative during the first week of the disease but becomes positive at the beginning of the second week and remains positive for months and years. A positive Widal, therefore, means that the patient either has or has had typhoid.

Pneumococcus agglutination is used to determine the type of pneumococcus invading the body in question after the organism has been isolated from the blood. If Type I is found, serum may be given to the patient. Type III is considered fatal.

**BLOOD MATCHING.**—Blood transfusion is used very frequently in pediatric practice in cases of sudden or gradual loss of blood. It has been found that the serum of a small percentage of individuals will clump the red blood cells of certain other individuals. In order to avoid any accidents, it is necessary to group or match the blood of the donor and of the recipient to ascertain that the serum of the patient would not agglutinate the corpuscles of the donor and *vice versa*. A number of methods have been described for blood grouping and blood matching. For practical purposes, the method of Lee or that of Kimpton will suffice.

*Method of Lee.*—A small amount of blood is collected from a patient (1 c.c. from the ear or finger is sufficient) and allowed to clot. The serum is then obtained. One drop of this serum is placed on a slide and mixed with a drop of a suspension of blood of the donor taken into 1.5 per cent citrate solution. (A few drops of blood are taken into approximately ten times the amount of 1.5 citrate solution and shaken. It is very important that the blood be dropped directly into the citrate, and should not be coagulated partially.) The result will appear in a few moments and is best examined under the microscope, where in the event of a positive test, marked agglutination will be evident. The reaction will also be evident macroscopically. In the event of a negative test it is a wise precaution to raise the cover-glass, and after making sure that the serum and cells are well mixed, to examine the preparation again. The only possible source of confusion is the appearance of rouleaux of the red corpuscles, indicating too thick an emulsion. If the test is negative, transfusion may be regarded as entirely safe.

*Method of Kimpton.*—Two to three drops of the donor's blood is collected into a small tube containing 0.5 per cent oxalate or salt solution (Solution 1). The oxalate prevents coagulation, thus keeping the corpuscles free.

Two to three drops of the donor's blood is collected into 0.5 c.c. of distilled water (Solution 2). This destroys the red blood cells and preserves the serum.

The above procedures are repeated with the recipient's blood. (Solution 3 = corpuscles and Solution 4 = serum.)

A drop of the donor's corpuscles (Solution 1) is now placed on a hang drop slide with a drop of the recipient's serum (Solution 4). The procedure is repeated with the donor's serum (Solution 2) and the recipient's corpuscles (Solution 3). The slide is observed under the microscope for clumping. Often clumping may be observed even with the naked eye. If clumping is present, the donor's blood cannot be used for transfusion.

Harp found that isoagglutination is rarely present at birth and during the first month of life. According to his findings, no test would have to be made on blood to be transfused to an infant during the first month of life.

**FRAGILITY OF BLOOD CORPUSCLES.**—Normally red blood cells do not hemolyze in salt solution above a dilution of 0.44 per cent. In hemolytic condition of the blood, the cells hemolyze with a higher salt solution even as high as 0.6 to 0.8 per cent.—in other words the cells are fragile. It is claimed that the type of jaundice can be differentiated by the fragility of the blood cells; hemolytic jaundice making the blood more fragile, obstructive jaundice producing no change in their resistance. The test cannot be considered specific, although it serves as corroborative evidence.

**WASSERMANN TEST.**—Although a positive Wassermann test may be obtained occasionally in disease other than syphilis such as in leprosy, jaundice or even in the early stages of scarlet fever, a 4-plus positive Wassermann test should be taken as an indication of a luetic infection, providing the test has been done with more than one antigen. A doubtful positive test should always be repeated. A negative Wassermann test does not exclude syphilis as the infection may be too recent in origin, may have subsided under treatment or may be localized in the central nervous system. In the latter case the cerebrospinal fluid Wassermann may be positive in spite of the negative reaction in the blood.

Occasionally a Wassermann test is negative in cases where clinical symptoms point to congenital or acquired syphilis. In such cases the test may be made more effective by giving the child a dose of neosalvarsan or several mercuric rubs, and the Wassermann repeated. Such a test is called provocative Wassermann.

## SAFETY OF LOCAL ANESTHETICS\*

WITH PARTICULAR REFERENCE TO COCAINE AND BUTYN

BY CARL NIELSEN AND JOHN A. HIGGINS, CHICAGO, ILL.

AN IDEAL local anesthetic for use in the practice of medicine and dentistry is one which combines universal efficiency with clinical safety, and which is easily soluble, capable of sterilization by boiling, and nonirritating. The professions are familiar enough with the unpleasant experiences with one or the other of the local anesthetics of the so-called "cocaine-group" that have been in common use for years past. This applies particularly to cocaine itself which, in spite of its many dangers, used to be the local anesthetic of choice until equally or more efficient but less dangerous products were developed for the purpose. The progress of recent years in this direction has advanced to the point where the local anesthetic is chosen with preference from among the group of non-habit forming synthetics often termed "substitutes for cocaine"; while cocaine itself is being regarded as a dangerous local anesthetic to be used only when no other is available. Sollmann<sup>1</sup> classifies all these anesthetics under the general term, "cocaine-group." They are all alkamine esters of aromatic acids, but the synthetic ones differ from cocaine in that they are not habit forming.

About two years ago, a new synthetic local anesthetic was introduced and described by Kamm, Adams and Volwiler, 1920,<sup>2</sup> and by Volwiler, 1921;<sup>3</sup> namely, para-amino benzoyl gamma di-n-butyl amino-propanol hydrochloride. This, as well as the succinate of the same base, was investigated pharmacologically by Bonar and Sollmann.<sup>4</sup> Our experiments include in addition to these salts also the much more soluble sulphate (Butyn). These extensive pharmacologic tests and the subsequent clinical trials established the superior efficiency of butyn to cocaine and procaine.

The first question asked, when such a new local anesthetic is introduced, is naturally, "How toxic is it compared to these?" The physician or dentist usually expects an approximate comparative figure in answer to the question, since unfortunately it often is customary to express the toxicity of local anesthetics in such figures without explaining how they are obtained. Although it has been repeatedly demonstrated that the ratio of toxicity of each of these local anesthetics varies greatly in the different species of animals, and that the toxicity of one and the same anesthetic in the individual species varies according to the concentration and mode of application, yet these arbitrary figures are very often applied as a direct measure for the clinical safety of the anesthetic in human therapy.

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Neither the efficiency nor the toxicity of a local anesthetic can be judged from a series of tests on a single animal species. Nor do the toxicity and side actions, as demonstrated on various laboratory animals, offer an approximate measure for the safety or non-safety in human therapy. The synthetic anesthetics, as a rule, present less variation and leave less uncertainty than cocaine in that direction.

TABLE I  
SUBCUTANEOUS INJECTIONS IN ALBINO RATS

The solutions used contained 10% of the anesthetics and 0.004% of epinephrin:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	370	1
Butyn	190	2

TABLE II  
SUBCUTANEOUS INJECTIONS IN RABBITS

The solutions used contained 5% of the anesthetics:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	200	1
Butyn	55	3-2/3

Bonar and Sollmann<sup>4</sup> in their work on new local anesthetics state: "The current methods of estimating efficiency, toxicity and irritation \* \* \* may render considerable practical service by cutting short the career of distinctly unpromising anesthetics before they are introduced into clinical practice. The final selection of the more valuable from the crowd of the more or less promising local anesthetics is much more difficult. \* \* \* Judgment must be cumulative since it cannot for the present be absolute." The safety of a "promising" local anesthetic can be judged only after long continued clinical experience with it.

We have, during the past five years, investigated a number of synthetic anesthetics prepared by Adams, Volwiler, Kamm and others in their endeavor to find one safer and more universally efficient than cocaine. Our work includes those investigated by Bonar and Sollmann; we also found butyn to be the most promising.

Bonar and Sollmann report the toxicity of para-amino benzoyl gamma di-n-butyl amino-propanol hydrochloride and cocaine as obtained by subcutaneous injections into albino rats and intravenous injections in cats and rabbits. Our investigations include aside from these the subcutaneous toxicity in rabbits, cats and dogs and the toxicity by stomach in cats; the action on blood pressure and respiration was also investigated. The following are extracts from our records, showing the vast differences in relative ratio of toxicity of these anesthetics, according to the animal used, and the mode of administration.

Similar series of tests were conducted with butyn solutions, previously steam sterilized for one hour or autoclaved half an hour at 15 pounds pressure. Neither the efficiency nor the toxicity was influenced by these pro-



cedures. In all these tables cocaine is used as a standard = 1 for the ratio of toxicity; it must be understood that the apparent variation in the ratios of toxicity of butyn expressed in this manner are due mainly to the great variation of toxicity of cocaine in these various animals.

This is conveniently expressed in the accompanying graphic illustration of the ratios of toxicity by the subcutaneous method. (Charts 1 and 2.)

It will be seen that butyn subcutaneously is twice as toxic on rats,  $3\frac{2}{3}$  times as toxic on rabbits, approximately equal in toxicity on cats, but slightly less toxic than cocaine on dogs. Introduced directly into the blood stream in rabbits and cats and orally to cats (Tables V, VI and VII) butyn is about

TABLE III

## SUBCUTANEOUS INJECTIONS IN CATS

The solutions used contained 15% of the anesthetics:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	50	1
Butyn	55	1

TABLE IV

## SUBCUTANEOUS INJECTIONS IN DOGS

The solutions used contained 15% of the anesthetics:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	45	1
Butyn	55	$\frac{4}{5}$

TABLE V

## INTRAVENOUS INJECTIONS IN RABBITS

The solutions used contained 1% of the anesthetics and were injected at the rate of 1 c.c. in 18 seconds:

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	12	1
Butyn	12	1

TABLE VI

## INTRAVENOUS INJECTIONS IN CATS

Bonar and Sollmann report the results obtained by Hatcher with para-amino benzoyl gamma di-n-butyl amino-propanol hydrochloride, this salt being termed "H" (butyn is the sulphate of the same base).

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	15	1
"H"	15 (approximately)	1

TABLE VII

## ORALLY IN CATS (ADMINISTERED IN GELATINE CAPSULES)

ANESTHETIC	M.F.D. IN MG. PER KG. BODYWEIGHT	RATIO OF TOXICITY COCAINE=1
Cocaine	40	1
Butyn	45	$\frac{8}{9}$

equal in toxicity to cocaine. It is evident that we cannot answer the question of safety to the human in terms of "toxicity" to experimental animals, since we would be as justified in saying that butyn is equal in toxicity or less toxic than cocaine as we are in stating that it is twice as toxic. The results in the larger animals are as good—if not indeed a better—guide to our judgment. No final conclusion as to the safety of a local anesthetic of this character to the human and the consequent extent of its usefulness, must be drawn from toxicity tests on animals. Such tests are, of course, of great value as preliminary indications, but they are apt to be misleading if confined to one or two species of small experimental animals. This is particularly true as long as we use cocaine, which is so inconsistent in its toxic effect on various animals, as a standard.

Butyn, for example, has a uniform ratio of toxicity subcutaneously in

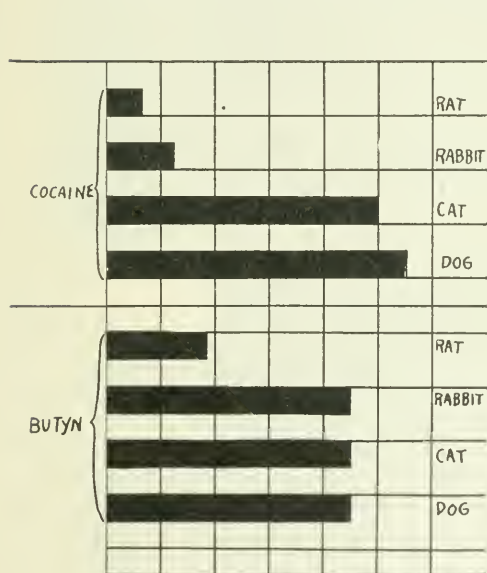


Chart 1.—Showing the comparative variation in toxicity of cocaine and butyn by subcutaneous injection in different animals.

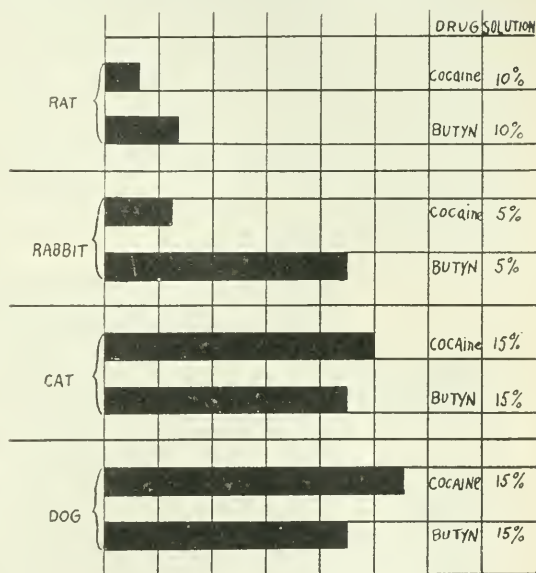


Chart 2.—Showing the comparative variation in toxicity of cocaine and butyn by subcutaneous injection in different animals.

rabbits, cats and dogs, while cocaine becomes more toxic in the same order, until in the dog it is more toxic than butyn. The toxicity of cocaine is more selective. Dixon<sup>5</sup> has shown that there is a definite relation between the amount of brain substance per weight of animal and the dose of cocaine required to produce convulsions. The convulsions are a result mainly of its action on the neurones of the brain. The higher the development of the brain, the more toxic its action. It requires 20 milligrams per kilogram bodyweight of cocaine to produce convulsions in the dog, but only 12 milligrams to produce a similar reaction in the ape. Our graphic cocaine chart is in conformity with Dixon's findings. In contrast, the butyn chart shows that the minimum fatal dose of this anesthetic is the same per kilogram animal in the rabbit, cat and dog. While cocaine, therefore, according to Dixon's observations, is more toxic to man than to animals, it is improbable that butyn would show great

variation—if any. The treacherous action of cocaine in the human on one hand and the degree of safety with which butyn has been extensively used clinically on the other, point in the same direction.<sup>6, 7</sup> So does the comparative action of the two on the blood pressure and respiration.

Figs. 1 and 2 show the effect of cocaine and butyn on the blood pressure and respiration in the same dog. The injections were 1 c.c. of a 1 per cent solution of each anesthetic with 30 minutes interval. Butyn was injected first. It will be seen that butyn caused only a very slight preliminary stimulation, then an abrupt fall for about a minute, followed immediately by a sharp rise to normal. There were no convulsions. Cocaine produced a considerable rise in pressure of short duration, then a gradual and long sustained

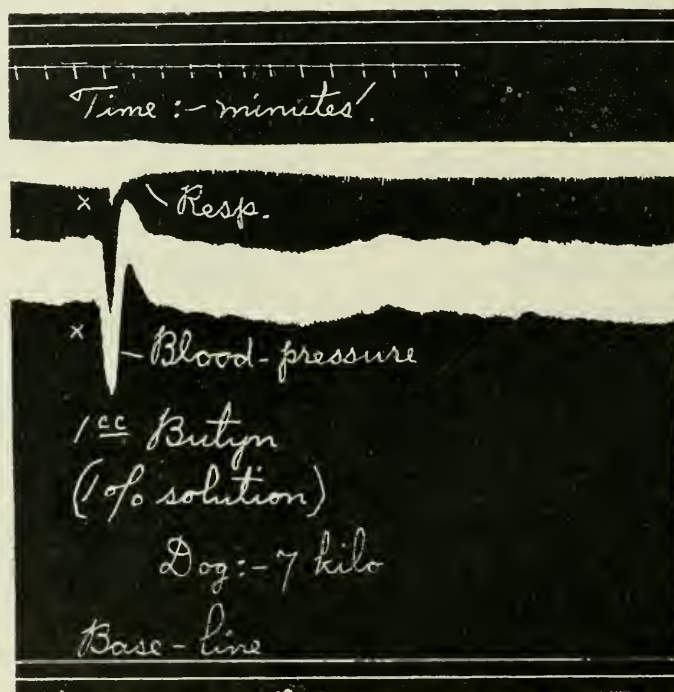


Fig. 1.

fall, accompanied by convulsions. Cocaine was also more depressing than butyn on the respiration.

In another dog (Fig. 3), the order of injections was reversed, the cocaine again showing a more prolonged depression. In a third and larger dog (Figs. 4 and 5) trichlorbutyl alcohol was used as an anesthetic and 5 c.c. of a 1 per cent solution of the local anesthetics were injected with a three hour interval. Again cocaine was more depressing than butyn. The reactions on this dog are not as distinct, probably due partly to the general anesthetic used. In comparing these tracings it should be considered that the amounts injected were the same for butyn and cocaine, although butyn as a local anesthetic, is at least twice as efficient as cocaine in all cases where these anesthetics are applied therapeutically.



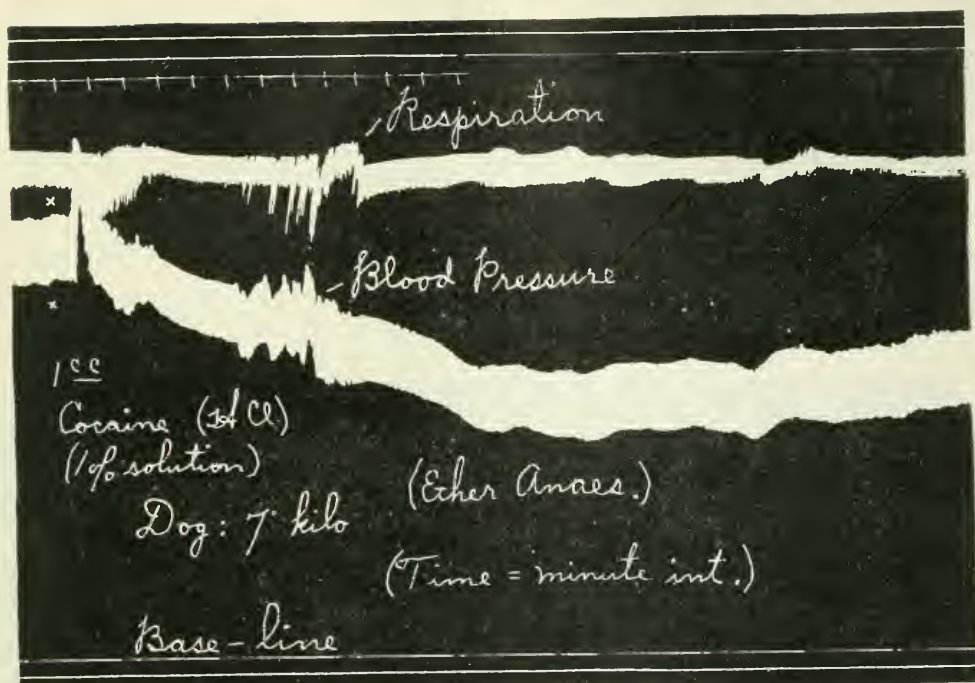


Fig. 2.

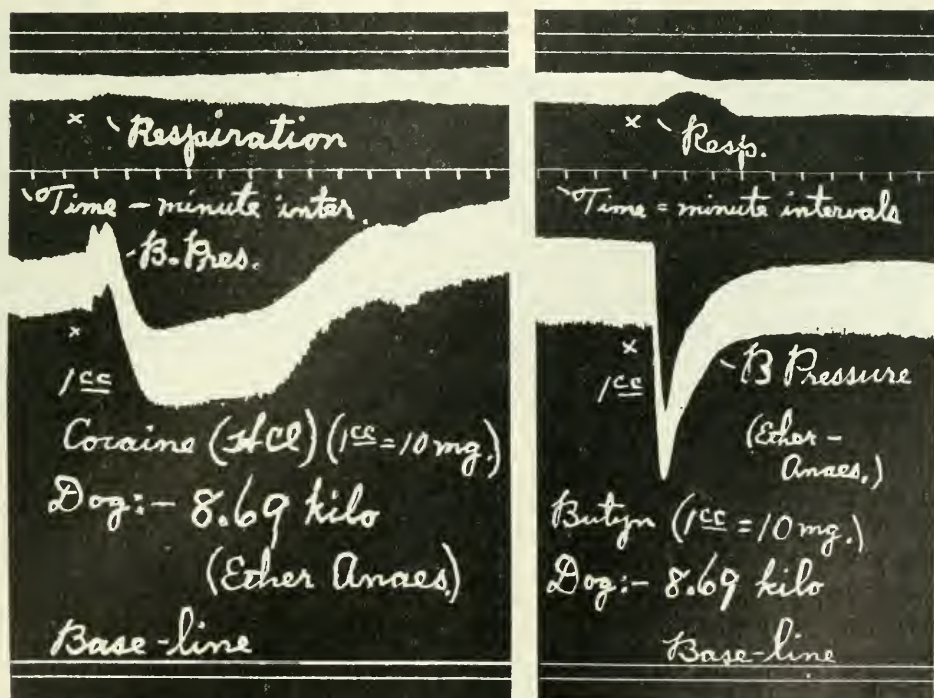


Fig. 3.



Undesirable side actions in patients treated with local anesthetics of this character will occur. They have been too frequent with cocaine and similar cases have been reported even from the use of procaine. Such cases are of course not always directly referable to the anesthetic, and may even be purely psychic in character. Butyn cannot be expected to be an exception to this rule. Years of clinical experience are necessary for proper judgment.

#### THE USE OF DRUGS IN THE TREATMENT OF POISONING BY LOCAL ANESTHETICS

Several methods of treatment for poisoning by local anesthetics have been proposed, but none of them have been very satisfactory.

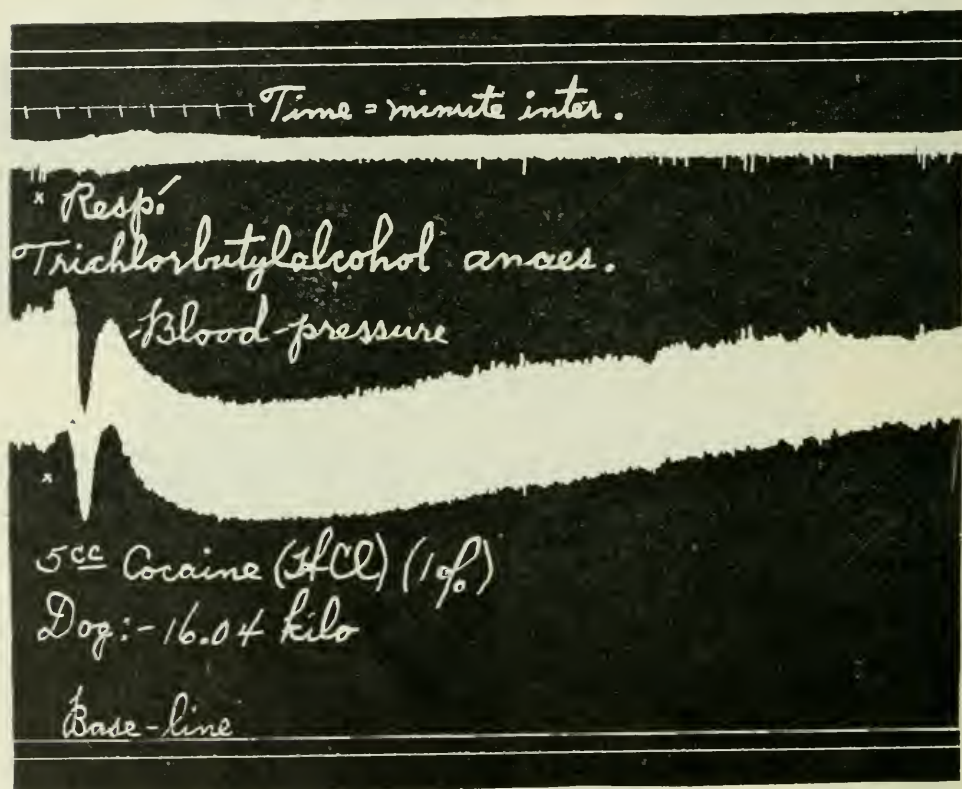


Fig. 4.

Sollmann suggests the following treatment of poisoning by local anesthetics: "If the drug has been taken by mouth, evacuation and chemie antidotes are indicated. If the anesthetic was injected, absorption should be checked by ligation, epinephrin or cooling. If symptoms have developed the head should be lowered; collapse treated by aromatic ammonia and caffeine; convulsions allayed by ether or chloral; and artificial respiration applied as needed; amyl nitrite has been recommended. Epinephrin and ouabain, intravenously, and cardiac massage are useful in animals (Eggleston and Hatcher, 1919), but have not been tried sufficiently in the clinic. Whatever delays death, is likely to save the life, in view of the rapid destruction of

these products. As prophylaxis the patient should be recumbent, if the operation permits. With nervous patients, it is advisable to inject morphine 15 minutes before the local anesthetic, and to delay the start of the operation till 20 minutes after the injection of the local anesthetic (Mayer et al, 1920)"

We have attempted to find a remedy which would not only prevent undesirable reactions from therapeutic and sublethal doses, but which would also eliminate convulsions and prevent death from fatal doses. The results of our work are reported in the following:

In the majority of experiments we used butyn as the local anesthetic. It was injected subcutaneously into rabbits and dogs in doses above fatal as well as in sublethal doses, sufficient to produce convulsions.

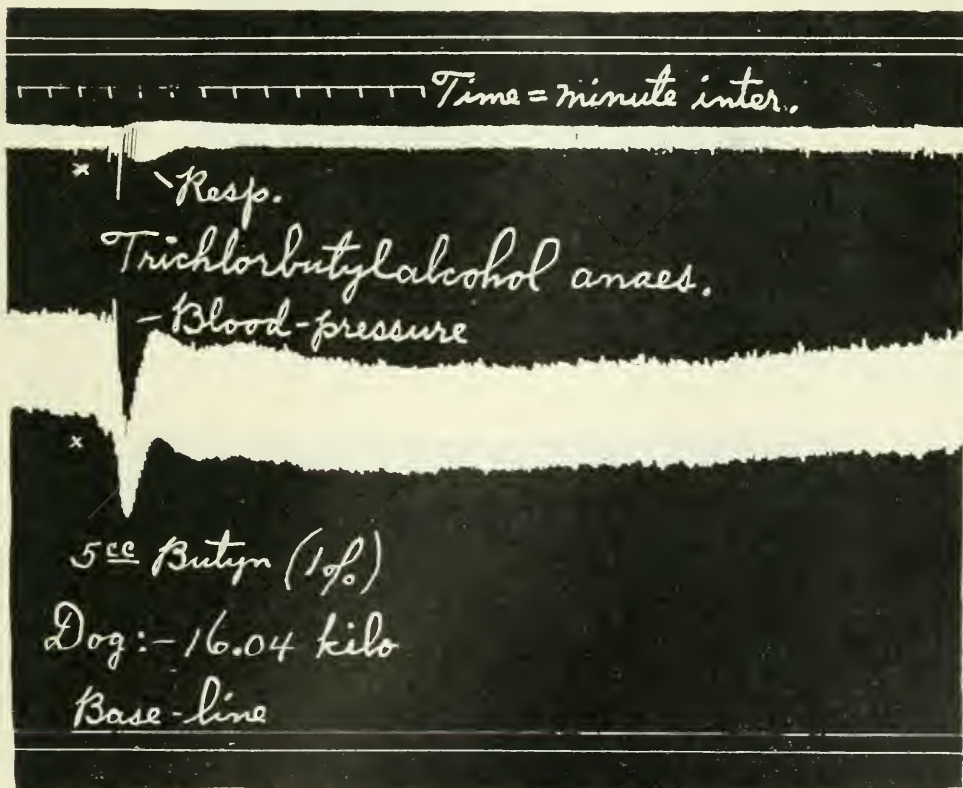


Fig. 5.

The following drugs were investigated for their detoxicating action:

Epinephrin  
 Caffeine Citrate  
 Hyoscine Hydrobromide  
 Morphine Sulphate  
 Atropine Sulphate  
 Hydrastinine  
 Pituitary Solution.

The use of pituitary solution was suggested by J. A. Higgins, and since it appeared to be more promising than the others it alone was subjected to further experiments, including its use in cocaine poisoning also.

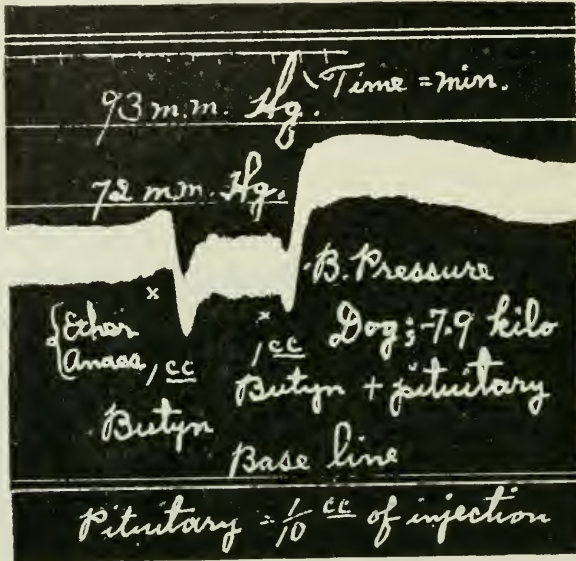


Fig. 6.

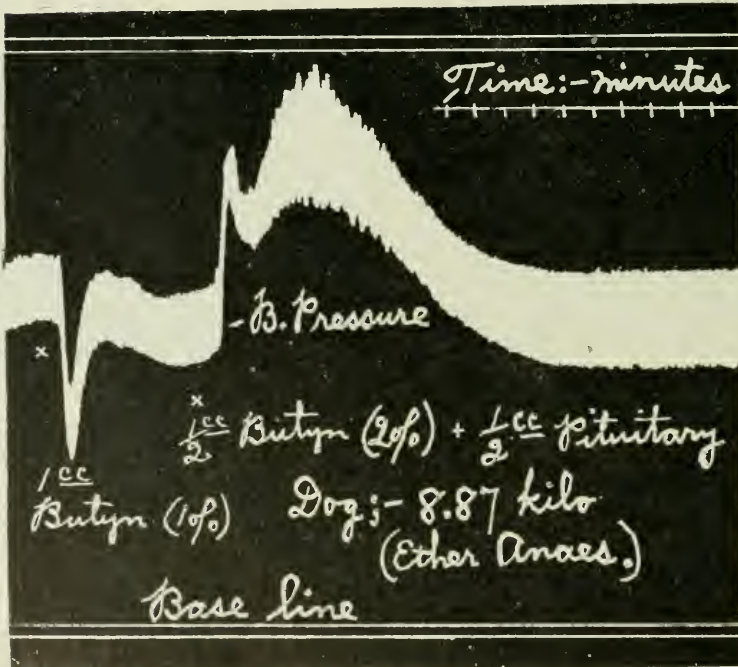


Fig. 7.



The pituitary solution we used was taken from samples sent over from our manufacturing department and found to be of the accepted standard activity on the isolated guinea pig uterus, and to produce the required sustained blood pressure rise in the dog.

The minimum fatal dose of butyn subcutaneously in rabbits and dogs is 55 milligrams per kilogram bodyweight (Tables II and IV). The minimum fatal dose of cocaine subcutaneously in dogs is 45 milligrams per kilogram bodyweight (Table IV).

Rabbit No. 2, weight 1.67 kg.; dose 55 mg. of butyn per kg. bodywt.; total dose 0.09185 grams. Total amount of a 5% solution 1.82 c.c. injected subcutaneously at 2:02 P. M. convulsions set in at 2:11 P. M. At the onset of convulsions,  $\frac{3}{4}$  of a c.c. of pituitary solution was injected intravenously. This injection immediately stopped the convulsions and the rabbit survived the fatal butyn injection.

Rabbit No. 3, weight 1.77 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1062 grams. Total amount of a 5% solution 2.13 c.c. injected subcutaneously at 11:25 A. M. Onset of convulsions at 11:28 A. M. Injected 1 c.c. of pituitary solution at 11:30 A. M. This injection checked the convulsions in a few seconds and the rabbit survived the otherwise fatal butyn injection.

Rabbit No. 4, weight 2 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.12 grams. Total amount of a 5% solution 2.4 c.c. injected subcutaneously at 1:41 P. M. Onset of convulsions at 1:49 P. M. Injected 1 c.c. of epinephrin solution 1:10,000 intravenously 1 minute after the onset of convulsions. The animal remained quiet for a short period after this injection, but convulsions returned and the rabbit died at 2:38 P. M. Postmortem examination revealed a straw colored serum flowing from the nostrils, a pinkish tinge around the mouth, congestion of the liver, heart veins distended; the lungs contained a small amount of serum, but there was no indication of a rupture of the lung vessels.

Rabbit No. 5, weight 2.05 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.123 grams. Immediately before injecting the butyn, 1 c.c. of a 1% caffeine citrate solution was given subcutaneously (at 1:02 P. M.). At 1:04 P. M. 2.46 c.c. of a 5% butyn solution was injected subcutaneously. The caffeine injection did not stop the convulsions and the rabbit died at 2:04 P. M.

Rabbit No. 6, weight 2.57 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1542 grams. At 1:55 P. M. a subcutaneous injection of  $\frac{1}{2}$  c.c. of pituitary solution was given, and immediately following this injection, 3.08 c.c. of a 5% butyn solution was administered subcutaneously. (1:57 P. M.) The rabbit lies down quietly. No convulsions occurred and the animal was apparently well recovered at 3 P. M.

Rabbit No. 7, weight 1.89 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1137 grams; total amount of a 5% butyn solution in 2.27 c.c. injected subcutaneously at 2:38 P. M. Convulsions began at 2:46 P. M., and an injection of 1 c.c. of 1% caffeine citrate solution was then administered intravenously. This injection did not stop the convulsions; the animal was still on its side at 4:50 P. M.; it recovered slowly.

Rabbit No. 8, weight 1.7 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.102 grams. In this rabbit the caffeine solution was administered immediately before the butyn (same as in rabbit No. 5). 1 c.c. of a 1% caffeine citrate solution was injected subcutaneously at 3:30 P. M. followed by a subcutaneous injection of 2.04 c.c. of a 5% butyn solution at 3:32 P. M. The caffeine did not prevent convulsions, but the animal survived the fatal dose of butyn and was apparently normal the following morning.

Rabbit No. 9, weight 2.02 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1212 grams. In this animal the caffeine dose was doubled. One c.c. of a 2% caffeine citrate solution was injected subcutaneously at 10:27 A. M., followed by a subcutaneous injection



of 2.42 c.c. of a 5% butyn solution at 10:28 A. M. Convulsions set in at 10:35 A. M., and the animal died at 10:41 A. M.

Rabbit No. 12, weight 1.35 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.081 grams. Immediately before injecting the butyn, the animal received 1/100 of a grain of hyoscine hydrobromide subcutaneously. Total amount of a 5% butyn solution injected subcutaneously 1.62 c.c. The animal had convulsions of a mild type, and recovered (3:45 P. M.) from the fatal dose of butyn.

Rabbit No. 13, weight 1.71 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1026 grams. Immediately before giving the Butyn injection the animal was given 1/4 of a grain of morphine sulphate subcutaneously. Total amount of a 5% butyn solution injected 2.05 c.c. The morphine did not prevent the convulsions and the animal died 14 minutes after the butyn injection.

Rabbit No. 14, weight 1.73 kg.; dose 60 mg. of butyn per kg. bodywt.; total 0.1178 grams. The animal was first given 1/100 of a grain of atropine sulphate subcutaneously, followed immediately by 2.36 c.c. of a 5% butyn solution subcutaneously. Some convulsive movements of a mild type; the animal tries to get up 47 minutes after the butyn injection. Recovered.

Rabbit No. 16 weight 1.745 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.1047 grams. The animal received first a subcutaneous injection of 1/64 grains of Hydrastinine followed immediately by 2.09 c.c. of a 5% butyn solution. The Hydrastinine did not check the convulsions but the animal survived the fatal butyn dose.

Rabbit No. 17, weight 1.575 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.0945. Immediately before giving the butyn injection, the animal received 1 c.c. of a 1:10,000 solution of epinephrin subcutaneously. Total amount of a 5% butyn solution injected 1.89 c.c. The epinephrin did not prevent the convulsions, but the animal survived the fatal butyn dose.

Rabbit No. 21, weight 2.17 kgs.; dose 60 mgs. per kg. bodywt.; total dose 0.1302 grams; total amount of a 15% solution of butyn 0.87 c.c. injected subcutaneously at 11:31 A. M. No convulsions occurred up to noon, probably due to faulty technic in the injection. At 12:02 P. M. 0.75 c.c. of a 15% solution was injected subcutaneously and at 12:11 P. M. convulsions set in. At this time 1 c.c. of a 1:1000 epinephrin solution was injected subcutaneously. The epinephrin did not prevent the convulsions and the rabbit died at 12:21.

Rabbit No. 18, weight 1.85 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.111 grams. The animal was given a subcutaneous injection of 2.22 c.c. of a 5% butyn solution at 4:35 P. M. At 4:44 P. M. convulsions started and at this time 1/100 of a grain of hyoscine hydrobromide was injected subcutaneously. This injection did not prevent the convulsions and the animal died at 4:52 P. M.

Rabbit No. 19, weight 2.65 kg.; dose 60 mg. of butyn per kg. bodywt.; total dose 0.159 grams. Total amount of an 8% butyn solution, 2 c.c. injected subcutaneously. At the onset of convulsions, 1 c.c. of pituitary solution was injected intravenously. The convulsions immediately ceased, the animal becoming completely relaxed and "flexible." Recovered.

It will be seen from these results that the detoxicating action of pituitary solution was far superior to that of the other drugs. When pituitary solution was given subcutaneously before a fatal dose of butyn was injected, there were no convulsions and the animals recovered. Administering the pituitary solution intravenously after the onset of convulsions from a fatal dose of butyn, it immediately stopped the convulsions and saved the animal from death. The results from these intravenous injections of pituitary solution were so striking and so unailing, that a series of experiments of this

character were demonstrated by one of us (J. A. H.) at the meeting of the National Anesthesia Research Society held in Cincinnati during the week of January 8, 1923. In order to find out whether or not pituitary solution injected subcutaneously after the onset of convulsions from a fatal dose of butyn would be of benefit also, the following experiment was made:

Rabbit No. 20, weight 3.34 kg.; dose 60 mg. per kg. bodywt.; total dose 0.2004 grams. Total amount of a 15% solution of butyn 1.34 c.c. injected subcutaneously at 11:05 A. M. Onset of convulsions at 11:13 A. M. at which time 1 c.c. of pituitary solution was injected subcutaneously. This injection did not stop the convulsions and the animal died at 11:21 A. M.

These experiments demonstrated the desirability of injecting pituitary solution simultaneously with the butyn. A series of dogs were therefore given subcutaneous injections of butyn in doses above the fatal and containing various amounts of pituitary solution. It was found that a relatively small dose of pituitary was sufficient to prevent convulsions and death. Still smaller doses of pituitary solution were sufficient to eliminate the symptoms ordinarily produced by high sublethal doses of butyn.

Dog No. 32, weight 9 kg.; dose 60 mg. of butyn per kg. bodywt.; total 0.54 grams. Total amount of a 15% butyn solution, 3.6 c.c., containing 0.09 c.c. of pituitary solution, injected at 11:17 A. M. No convulsions occurred. There was some incoordination and some excitement. Animal recovered.

Dog No. 33, weight 8.73 kg.; dose 30 mg. of butyn per kg. bodywt.; (high sublethal dose); total dose 0.2619 grams; total amount of 15% butyn solution, 1.75 c.c., containing 0.022 c.c. (approximately 1/45 c.c.) of pituitary solution injected subcutaneously at 1:55 P. M. Bowel movements and vomiting at 2:07 P. M. Animal somewhat excited. Vomits again at 2:30 P. M. Bowel movements at 2:35 P. M. Dog somewhat nervous. No convulsions. Recovery at 3:30 P. M.

Dog No. 34, weight 4.48 kg.; dose 40 mg. of butyn per kg. bodywt. (high sublethal dose); total dose 0.1792 grams. Under ordinary circumstances this dose produces violent convulsions. Total amount of a 15% butyn solution, 1.194 c.c., containing approximately 1/100 of a c.c. of pituitary solution per kg. bodywt. (Total dose of pituitary solution 0.05 c.c.) injected subcutaneously at 10:30 A. M. No convulsions; animal is perfectly quiet; no excitement. Recovery at 12:15. The animal did not refuse food offered at this time (in contrast to animals who have received high sublethal doses of cocaine, who always refuse food for a long period of time after apparent recovery.)

In view of these excellent results from the use of pituitary solution in treating poisoning by butyn, a few experiments were conducted to show whether it would be beneficial also in cocaine poisoning; if so, it would seem highly probable that pituitary solution would be of equal benefit in the treatment of poisoning by all the anesthetics of this group.

Dog No. 35, weight 5.02 kg.; dose 25 mg. of cocaine per kg. bodywt. This is a high sublethal dose which always produces great excitement, incoordination, extreme anxiety, followed by very violent convulsions. Total dose 0.1255 grams. Total amount of 15% cocaine solution, 0.84 c.c., containing approximately 1/100 of a c.c. of pituitary solution per kg. bodywt. of animal (total dose of pituitary solution 0.050 c.c.). Injection made at 10:50 A. M. Incoordination a few minutes after the injection; the animal was somewhat excited, but no convulsions occurred. Animal apparently recovered at noon, but refuses food.

Dog No. 36, weight 4.82 kg.; dose 50 mg. of cocaine per kg. bodywt. This dose is above fatal. (M. F. D. subcutaneously in dogs 45 mg. per kg. bodywt.) Total dose 0.241 grams. Total amount of a 15% cocaine solution 1.61 c.c., containing approximately 1/100 of a c.c. of pituitary solution per kg. bodywt. of animal. Total dose of pituitary solution 0.05 c.c. A few minutes after the injection there was an increase in saliva; the animal became extremely excited; pupils very dilated. Animal turning its head unceasingly from one side to the other in extreme anxiety; marked incoordination; muscular weakness of hind quarters; animal fell over on one side and was unable to arise. At 12:30 the animal attempted to get up, but was too weak in the hind limbs. Later it finally succeeded in getting up and at 1:30 P. M. the animal moved about in an excited condition; the flow of saliva decreased; the animal refused food; there were some twitching movements of the head, but no convulsions. Animal recovered.

The action on the blood pressure of butyn solution and pituitary solution mixed compared to that of butyn solution alone, is shown in Figs. 6 and 7. In Fig. 6 the dog was given an intravenous injection of 1 c.c. of a 1 per cent butyn solution, producing as usual an abrupt fall in blood pressure. Five minutes later another 1 c.c. of a 1 per cent butyn solution, containing 1/10 of a c.c. of pituitary solution was given; this injection caused only a very slight fall of blood pressure followed by an immediate high and sustained rise.

In Fig. 7 a dog received 1 c.c. of a 1 per cent butyn solution intravenously, producing an immediate abrupt fall in blood pressure; a few minutes later an injection of 1/2 c.c. of a 2 per cent butyn solution mixed with 1/2 c.c. of pituitary solution was injected intravenously and this injection caused an immediate and high rise of blood pressure. The blood pressure did not fall below normal thereafter.

#### CONCLUSIONS

The safety of a local anesthetic in human therapy should not be judged from its toxicity compared to cocaine on experimental animals.

In determining the toxicity of a local anesthetic on animals, as many different species as possible should be investigated, preferably higher experimental animals. The toxicity should not be measured by comparison with cocaine as a standard without taking into account the fact, that cocaine is highly selective in toxicity, that is, the higher the development of the brain in the animal, the more toxic is the cocaine. The comparative variation in toxicity depending upon the species of animal, mode of application, concentration of solution, rate of injection, rate of absorption, rate of destruction in an excretion from the body, as well as the comparative action on blood pressure and respiration, should be determined.

With these pharmacologic data in mind, final judgment on the safety and the field of usefulness of a promising local anesthetic in human practice, should be passed only after sufficient clinical experience.

Various drugs were investigated for their detoxicating action in the treatment of poisoning by the local anesthetics of the so-called "cocaine group" (alkamine esters of aromatic acids). Pituitary solution appeared to be superior to the other drugs investigated, and holds out promise both as a preventive of undesirable symptoms from sublethal doses as well as an anti-

dote in poisoning by fatal doses of these local anesthetics. Judging from our experiments, small therapeutic subcutaneous doses of pituitary solution injected simultaneously with the local anesthetic are sufficient to eliminate the fall in blood pressure from therapeutic doses as well as the convulsions from higher doses, and death from fatal doses. Used as an antidote in cases where the convulsions have already set in, pituitary was effective only when administered intravenously. It deserves clinical trial. It should, of course, not be used in pregnant or in high blood pressure patients.

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## ATRESIA OF THE PULMONARY ARTERY IN A CONGENITALLY DEFECTIVE HEART\*

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CONGENITAL heart disease, a condition first discussed by Senae in 1749, involves the existence of anomalies in the anatomical structure of the heart or great vessels, of intrauterine origin, leading to disturbances in the circulation. Every case, especially if it is one of the rarer varieties, should be recorded, since the congenitally defective or malformed heart is an object of widespread interest to the anatomist and embryologist, as well as to the pathologist and clinician. Recently an example of the rare congenital cardiac condition, pulmonary atresia, was observed at necropsy in a four and one-half months old female infant. The following defects were present: Patency of foramen ovale, ventricular septum, and ductus arteriosus; atresia of pulmonary artery and valve and conus pulmonalis; origin of aorta from right ventricle; complete cleft palate and right hare lip and mobile cecum.

### CASE HISTORY

Case 1 (A395079). The child, aged two and one-half months, was admitted to the Worrell Hospital June 16, 1922, for plastic operation for harelip on the right and complete cleft palate. Her father, a physician, said that she was the third of three children, the other two being well formed, and healthy. The child's birth had been uneventful. She weighed seven pounds at birth, but had gained very little since; for the first three weeks she was very cyanotic and continued to have spells of strangulation and cyanosis. Her weight at the time of examination was about 8 pounds, she was emaciated and the skin was loose and flabby. The left upper eyelid was shortened, causing extreme difficulty in opening the eye; the left eyeball was about 60 per cent normal in size, but was perfect in function and by objective examination. There was some nystagmus at times on looking to the left. The sagittal suture extended quite far down on the forehead. The veins of the upper abdomen were distended. Occasionally the child breathed slowly, at which time a continuous hum was heard over the base of the heart and in the pulmonary area, rapidly diminishing toward the left and apex. The manubrium sterni was somewhat prominent. Four days after the first examination the color was better. The erythrocytes numbered 4,880,000; the leukocytes 6,900, and hemoglobin was 80 per cent. The urine was normal.

August 9, 1922, the patient was operated on for cleft palate. There was marked cyanosis and straining before ether was taken, but she did well under it and the post-operative recovery was uneventful. August 16, 1922, the operation for harelip was performed. The temperature rose to 103 after operation and remained there. There was considerable cyanosis, and death occurred suddenly about fifteen hours after the operation.

At necropsy, findings as follows were noted: The body was poorly developed and poorly nourished, 56.5 cm. in length. The left eye was smaller than the right; the iris of the left eye was 7 mm. in diameter; of the right eye, 10 mm.; the pupil of the

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left eye, 1 mm.; of the right eye, 1.5 mm. There was considerable cyanosis about the face, lips, and neck. The cecum and ascending colon were abnormally mobile, and were located just to the right of the middle line. The ductus venosus was closed at its portal and hepatic ends. The middle and lower lobes of the right lung were slightly collapsed, and somewhat flabby in consistency; a small cut section readily sank in water, although the entire mass of lung tissue floated, together with the heart. Otherwise both lungs appeared to be normal.

The right auricle was enormously dilated. The superior vena cava and all the coronary vessels were distended with blood. The cardiac muscle was bright red and firm. The wall of the right ventricle was markedly thickened, averaging 0.5 cm.; the papillary muscles were correspondingly large and prominent (Fig. 1). The depth of the right ventricle was 2.5 cm. In the upper part of the interventricular septum there was an oblique oval aperture, 11 by 3 mm. All that remained of the conus arteriosus and the pulmonary valves was a minute depression 4 mm. in length and 1 mm. in

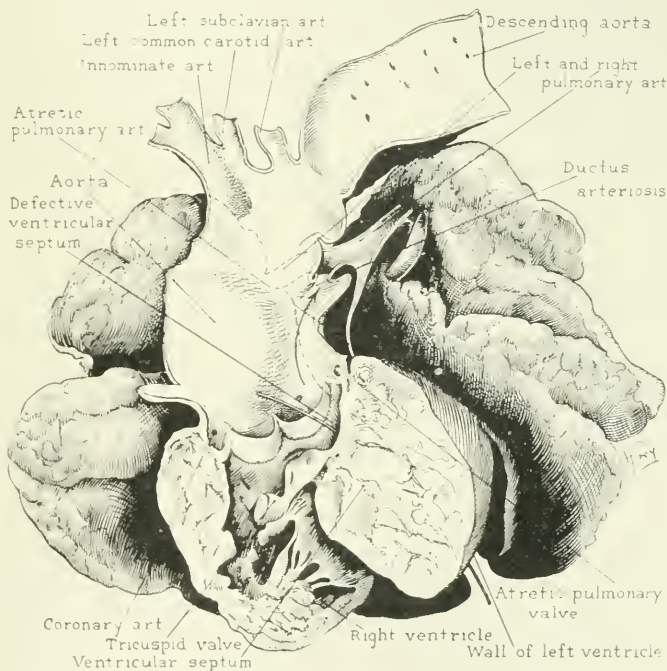


Fig. 1.—Posterior view of heart and lungs.

width, in the wall of the right ventricle, to the right and at the base of the interventricular septum, beside the opening in the septum, and on a level with the aortic cusps. In this shallow depression could be made out six oblique, minute subdepressions, as well as two above them. The whole mass was surrounded by a smooth, muscular, elliptical "plateau." About 4 mm. above this a narrow fibrous cord 0.5 mm. in diameter, entered into the muscle at the top of the ventricular septum and ended blindly, being lost in the muscle mass. This cord was barely dissectable grossly, had a minute lumen which passed over in the fibrous cord for 8 mm. to expand and communicate definitely with the right pulmonary artery, at its point of juncture with the latter, the circumference of the channel being 4 mm. The right and left pulmonary arteries presented normal lumina of 10 mm. circumference. The left pulmonary artery, near its point of juncture with the right, was connected with the arch of the aorta, opposite the arc from which the head vessels were given off, by a duct 5 mm. in length and 8 mm. in circumference, with slightly roughened intimal lining, the patent ductus

arteriosus. The aorta opened directly out of the right ventricle, and measured 36 mm. in circumference at its root. Three delicate, competent aortic valves were present, together with normal openings of the right and left coronary arteries. The aorta maintained its large circumference until the head vessels and ductus were reached, beyond which it presented a definite stenosis, being reduced to 15 mm. in circumference. One cm. beyond, it again widened to a circumference of 30 mm. The head vessels passed off normally. Two mm. below the origin of the left subclavian a minute accessory vessel originates. The paired intercostal arteries arise normally. The aorta at its point of stenosis presented a somewhat roughened intima, with a slightly elevated transverse ridge distally. Another "V"-shaped elevation occurred at the point of separation of the ductus and the narrowed aorta; and a transverse ridge stretched across the left pulmonary artery directly opposite the point of entry of the ductus.

The foramen ovale was patent (Fig. 1), presenting a complete opening, 10 by 3 mm. The openings of the coronary sinus, the superior and inferior venae cavae were in their normal positions. The wall of the right auricle and the auricular appendage were excessively hypertrophied. The tricuspid valve measured 4.3 cm. in circumference and presented a delicate line of closure. The pulmonary veins opened normally into the left auricle. The mitral valve was delicate and competent, and measured 3.1 cm. in circumference. One of its cusps was continuous through the defective ventricular septum with one of those of the tricuspid valve. The depth of the left ventricle was 2.3 cm.; the average thickness of its wall was 0.5 cm., small as compared with that of the right ventricle.

#### DISCUSSION OF THE LITERATURE

In 1880 Stifel collected forty-six cases of pulmonary atresia from the literature. In 1898 Vierordt added twenty-four cases, bringing the total to seventy, although there may be some overlapping of the two series. The nine cases reported in the literature since 1898 bring the total number of cases of pulmonary atresia to seventy-nine. Vierordt estimated the minimum number of reported cases of pulmonary stenosis to be three hundred.

The first paper on congenital atresia of the pulmonary artery was written by Hunter in 1783. There followed at infrequent intervals reports of isolated cases, until in 1858 Peacock published his classic on cardiac malformations. Among one hundred eighty-one instances of congenital heart disease which he assembled, one hundred nineteen (66 per cent) presented pulmonary stenosis or atresia (ninety, stenosis, twenty-nine, atresia). Rauchfuss' series of 1878 included eighty-one of stenosis and thirty-three of atresia; Vierordt's series, eighty-three of stenosis and twenty-four of atresia. In 1891 von Etlinger collected two hundred sixty-six cases of stenosis and atresia.

Stifel made a thorough study of pulmonary atresia in 1880. He found forty-six cases recorded to date, in six of which the pulmonary artery was represented by a solid, fibrous cord; in thirty, the pulmonary orifice was completely closed, and in ten, the obliteration was indefinite. Thirty-five were accompanied by a defective ventricular septum, thirty-eight by a patent ductus arteriosus, and twenty-eight by a patent foramen ovale. Eleven cases of the series showed dextroversion. Fifty per cent of the patients died within the first three months, 74 per cent within the first year, 90 per cent within the first twelve years, while one survived to the age of thirty-seven. Hirschfelder states that 78 per cent of the patients with atresia die during the first year, while only 9.7 per cent of those with stenosis die during the same period,



although 64 per cent die before puberty. Rauchfuss places the average duration of life in pulmonary stenosis at nine years, and in pulmonary atresia at three years.

Maude Abbott has analyzed six hundred thirty-one cases of congenital heart disease, with postmortem reports all from reliable sources, thus presenting a fairly accurate basis for a statistical study. The series includes ninety cases of pulmonary stenosis and twenty-nine of pulmonary atresia. Of the latter the ventricular septum was defective in 72 per cent; the ductus arteriosus and the foramen ovale were patent in 65 per cent.

Eleven of twenty-four of these atresia cases had defective ventricular septum and patent foramen ovale, the same combination of defects being present in the case reported here. The ages of patients ranged from thirteen days to thirteen years. Five were males, two were females. Seven had patent ductus arteriosus; seven had dilated aortas; nine, dextroversion of the aorta; seven, hypertrophy of the right auricle; eight, hypertrophy of the right ventricle; and two, hypertrophy of the left ventricle. In six cases there was marked cyanosis; in two, moderate; and in one, slight. In four, there were delayed development, systolic murmur, and dyspneic attacks; in three, clubbing of fingers; in two, thrill, pulsation, and dyspnea; and in one, precordial bulge and increased second pulmonic sound. Hence in the entire series of cases collected (to 1908) there are but seven which resembled the case I report here, that is, with defective ventricular septum, patent foramen ovale and ductus arteriosus, and atresia of the pulmonary artery. Of the twenty-one cases with defective ventricular septum, the aorta arose from both ventricles above the opening in seven, and entirely from the right ventricle in fourteen.

In the same article Abbott describes a specimen from the McGill Pathological Museum, presented by Sir William Osler, which closely resembles the one I have described.

In recent years occasional cases of pulmonary atresia have been recorded in the literature, such as those by Grothe in 1898, Curl in 1905, McCrae 1906, and Burnier in 1907. In 1915 Stoddard collected four cases that had been reported by D'Espine in 1908, Bach in 1909, Vallois in 1910, and by Edwards in 1911. Edwards emphasizes the fact that in complete pulmonary stenosis, or atresia, the blood reaches the lungs by one of five routes: (1) patent foramen ovale, (2) defective ventricular septum, from the right into the left side of the heart, and by way of the aorta, (3) through the patent ductus arteriosus, (4) the bronchial arteries, or (5) the anomalous branch of the left subclavian artery (one instance). Carpenter describes an aorta arising from both ventricles (four-fifths from the left), a small pulmonary artery (0.31 cm. in diameter) with two semilunar valves, a patent ductus arteriosus, the semilunar valve efficiently guarding the foramen ovale, but displaying a tiny perforation, in a ten weeks' child, without cyanosis. Bach's case of seven weeks showed cyanosis, and the pulmonary artery arising abruptly from the heart muscle, the presence of stenosis and insufficiency of tricuspid



valve, persistent foramen ovale and ductus arteriosus. The walls of the right ventricle were very thick and the cavity was rudimentary.

D'Espine reports a case of "cyanose congénitale paroxystique," the fifth on record, a condition first described by Variot, as an intermittent congenital cyanosis with periods of freedom, or near-freedom, and caused by "hypertrophy of the right ventricle; communication of aorta with right ventricle; stenosis of conus pulmonalis; and an interventricular communication." In Burnier's case the pulmonary artery terminated blindly in the heart. D'Espine notes that the pulmonary artery and valves are present, but very small. He believes the predisposing cause of the cyanosis to be the mixture in the aorta of the two blood streams which have come from the two ventricles. The right ventricular hypertrophy in these cases causes an increase in the proportion of dark venous blood in the aorta, and so brings about a latent predisposition toward cyanosis. Stasis of blood brought about suddenly by an acute dilatation of the right ventricle due to disease, muscular fatigue, emotion, digestive influences, and so forth, can easily disturb the delicate equilibrium and convert latent into active cyanosis, since insufficient blood then passes up the aorta, through the ductus, and into the pulmonary circulation. During these paroxysms of cyanosis there are often present edema of the eyelids or extremities, great increase of percussion dullness to the right, and precordial heave.

From an analysis of 242 necropsies in cases of congenital heart disease, Holt and Howland (1922) find that a defective ventricular septum is the most common lesion, occurring in 149 cases, although it is the sole lesion in only five of these. It is most common in association with pulmonary stenosis or atresia, namely in ninety-two cases.

#### GENERAL DISCUSSION

Pulmonary stenosis and pulmonary atresia must be differentiated sharply, although they represent merely different degrees of the same pathologic process. Stenosis is a narrowing without obliteration; atresia is a narrowing, with obliteration, of the functional channel. Pulmonary stenosis is the most common variety of congenital heart lesion, and is from three to eight times as common as atresia.

As to the cause of atresia of the pulmonary artery, two possibilities present themselves: Endocarditis in fetal life is applicable in such a limited number of instances that practically it may be dismissed. Osler could not imagine "a fetal endocarditis localized to so small an area as the pulmonary valves must be before the eighth week of fetal life." The second possibility, defective development of the pulmonary artery, has much to support it. At about the eighth week of fetal life the pulmonary artery becomes separated from the truncus arteriosus together with the sixth branchial arch on the left side, the communication with the descending aorta persisting as the ductus arteriosus. The truncus pulmonalis, because of the twisting of the cardiac tube, protrudes ventrally, while the aortic portion lies dorsally. The truncus pulmonalis thus represents the ventral half of the truncus arteriosus

and springs directly from the infundibulum of the right ventricle. Stenosis or atresia may occur as a result of retarded development in the infundibulum below the valves, at the valves, or in the pulmonary artery itself. Atresia is caused by unequal division of the truncus arteriosus by the septum of the bulb, which normally presents its convexity downward toward the pulmonary half of the truncus, approaches the truncus, and fuses with it as far down as its mouth. The cause of the unequal division is probably imperfect development of the fifth branchial arch.

His claims that such disturbances of developmental conditions are brought about by "insufficient nourishment, insufficient aeration of blood, and mechanical causes resulting from malpositions of uterus, disturbed placental circulation, and so forth." Panum has shown them to be producible experimentally in birds by raising the temperature of incubation, or by inducing fever in the mother. Keith, as a result of a series of personal observations on conus stenosis and the study of two hundred seventy malformed hearts in the museums of London, has advanced the view that cardiac defects are nearly always developmental in origin, and that pulmonary stenosis is usually due to subinvolution of the bulbus cordis, the development being arrested at a stage when this fourth primitive chamber existed in the heart.

The course of the circulation of the blood in the heart in the case here reported must be as follows: From the right ventricle into the aorta, through the ductus arteriosus into the pulmonary arteries, back through the pulmonary veins into the left auricle and ventricle, through the defective interventricular and interauricular septums into the right ventricle and auricle and also from systemic veins into the right auricle and ventricle. This explains the great bulk of work thrown on the right side of the heart, and its subsequent hypertrophy and dilatation. The left side is practically useless except as a passageway.

Why is the aorta given off from the right ventricle? Because in these cases of atresia, practically all of which show this phenomenon of dextroversion (*Rechtslage*), the deflection of the ventricular septum to the left is so great that as a result the aorta lies in the axis of the right ventricle.

The symptoms which may be expected in such cases are briefly: cyanosis, also known as the morbus coeruleus or blue sickness, shown at birth in seventy-four of Peacock's one hundred one cases and in practically all within one or two years; dyspnea, especially on exertion, headaches, cold hands and feet, and a tendency to bleed easily. During the attacks of strangulation the patient becomes blue or black, and a fainting spell or epileptiform convulsion may result from the venous stasis and cerebral ischemia. There is a precordial bulge, a wavy systolic impulse, increased percussion dullness on both sides, decreased or absent second pulmonic sound, a rough systolic thrill in the pulmonic area together with a murmur, transmitted up and to the left, but not into the vessels of the neck. The liver and spleen may be large. A polycythemia of six to nine billion is the rule, with a hemoglobin of 110 to 130 per cent. Secondary lesions often exist, such as distention of the arteries and veins, and malformation of arterioles and venules. The former results

in stasis, edema, frequent hemorrhages, and retarded growth in stature and intelligence; the latter produces terminal capillary sinuses in the skin viscera, and retina (diagnosed by ophthalmoscopic examination). Clubbing of the fingers is often present and is occasioned by excessive connective tissue proliferation as a result of the venous stasis.

The case presented seems to be unique in the combination of associated cardiac anomalies displayed, and remarkable in the comparative normality of the child aside from the congenital hare lip and cleft palate. The cyanosis was of such a remittent type, associated with periods of comparative freedom, as to justify placing this case in D'Espine's group of "cyanose congénitale paroxystique." Although the exact number of cases of pulmonary atresia recorded to date cannot be determined accurately, this may be called the eightieth.

#### SUMMARY

A case of congenital atresia of the pulmonary artery, pulmonary valve, and infundibulum of the right ventricle is described in conjunction with other developmental anomalies: origin of the aorta from the right ventricle; presence of a patent ductus arteriosus, and defective ventricular and auricular septums; complete cleft palate and right hare lip. The ascending aorta, as far as the opening of the ductus arteriosus, thus served as both a pulmonary artery and an aorta, the ductus arteriosus conveying blood from the aorta to the lungs through the widely patent right and left branches of the atretic pulmonary artery. The child lived four and one-half months in fairly good health, and died only after a plastic operation. Prior to operation she had shown intermittent cyanosis and attacks of strangulation.

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## THE INCREASE IN VIRULENCY OF NONPATHOGENIC MICRO-ORGANISM BY CHEMICAL SUBSTANCES\*

BY R. H. LEE, B.S., AND LLOYD ARNOLD, M.D., CHICAGO, ILL.

HANS MUCH<sup>1</sup> records some observations made with nonpathogenic bacteria that were apparently changed into a pathogenic type by simultaneously injecting in the experimental animal a weak solution of lactic acid.

It has been known for some time that lactic acid increases the virulence of some of the pathogenic microorganisms. This has been explained more upon the basis of an impairment of the defensive mechanism of the host than upon an actual change in virulency of the invading bacteria. Gabritschevsky<sup>2</sup> found lactic acid to be indifferent to or possibly exerting a "negative chemotaxis" on leucocytes.

In the experiments reported by Much there was an actual change from a typical nonpathogenic or avirulent to a pathogenic or virulent type. *Bac. mycoides*, *B. proteus* and even *B. subtilis* caused acute infections with early death, and the respective organism could be isolated from the blood and organs after death. The strains Much used were isolated from the air and contaminated medium in his laboratory. He used mice, guinea pigs and rats. The lactic acid was in 0.01 to 1.0 per cent concentrations. For mice 0.2 c.c. of the dilute lactic acid was used. This was injected at the same time as the nonpathogenic microorganism, either in the same place or in a different place. If dead bacteria were used no change was produced. His experiments with *B. subtilis* injections combined with lactic acid were negative, but when lactic acid was added to bouillon, to make a concentration of 1.0 to 0.001 per cent lactic acid in bouillon and *B. subtilis* was grown in this lactic acid bouillon, it became very virulent for mice. Much claimed this artificially produced virulence was caused by the action of the lactic acid on the living microorganism.

Bachmann<sup>1</sup> repeated the experiments recorded by Much, but found that the lactic acid injection had no effect at all on the microorganisms. This author used *Proteus vulgaris*, *Proteus X19*, *B. subtilis*, *B. typhosus*, *B. shiga* and *kruse*. The experimental animals used were mice and guinea pigs. The results were all negative. Bachmann thinks probably "dass die vom Much erzielten Ergebnisse an besondere biologische Eigenschaften seiner Kulturen geknuepft sind, die erst unter dem Einfluss des Milchsaur-zusetzes in die Erscheinung treten." Lange and Yoshioka<sup>3</sup> repeated the experiments of Much with negative results.

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## TECHNIC

Plain Liebig peptone (Fairchild's) bouillon was used as fluid medium. 2.5 per cent agar was added to make solid medium. *B. subtilis* was an old laboratory strain. *B. proteus* was obtained from Prof. Kendall.

In order to grow each of these microorganisms in a bouillon containing lactic acid, we determined the maximum amount of the lactic acid that could be added to the bouillon that would support growth of the respective bacteria. 0.17 per cent lactic acid in bouillon was the maximum concentration for *B. subtilis*; even with this amount, the third 24 hour transplantation showed a poor growth, it was then transferred to plain agar to rejuvenate the culture and after 24 hours' growth again transplanted to the lactic acid bouillon. The *B. subtilis* was kept going in this way for three weeks before the animal inoculations were started.

It was found that 0.08 per cent concentration of lactic acid in bouillon was all that would support growth of the *B. proteus* strain. It was transferred to plain agar every third day, as was done with the *B. subtilis*, to keep the growth of uniform density as compared with the plain bouillon control tube. This was also carried on for three weeks before animal inoculations were started.

The streptococci used were hemolytic and nonhemolytic strains isolated from the throats of patients suffering from diphtheria, also some strains from normal throats were used. No effort was made to grow the streptococci on lactic acid bouillon.

## EXPERIMENTAL

*B. subtilis*.—One c.c. of a 24 hour culture of *B. subtilis* in 0.17 per cent lactic acid bouillon; 1 c.c. of a 24 hour culture of *B. subtilis* and 1 c.c. of 0.85 per cent lactic acid were injected subcutaneously in the back near the tail in two different mice. One c.c. of a 24 hour culture of *B. subtilis* in plain bouillon and 1 c.c. of 0.85 per cent lactic acid were injected separately in the same manner in two other mice; these served as controls. Four mice were used in this experiment. This was repeated six times, making a total of 24 mice in the whole series. All mice were alive after 30 days' observation.

*B. proteus*.—We used the same procedure with *B. proteus* as was used with *B. subtilis*. Six groups of 4 mice each were injected. One control *B. proteus* mouse died after 24 hours, this bacillus was recovered in pure culture from the heart's blood. The remaining 5 mice were living 30 days after injection. The lactic acid control mice were not affected. One of the 6 mice injected with *B. proteus* grown in lactic acid bouillon died three days after injection, *B. proteus* was recovered from the heart's blood. Three of the 6 mice injected with both *B. proteus* and lactic acid died, two 24 hours and one 3 days after injection. *B. proteus* was isolated from heart's blood in the one that died 3 days and in one of the two that died within 24 hours.

A 24 hour bouillon culture of *B. proteus* was killed by heating to 58° C. for 1 hour. One c.c. of this was injected subcutaneously in the same manner into each of six mice, into three of these 6 mice was also injected 1 c.c.

of a 0.85 per cent lactic acid. All of the mice injected with dead *B. proteus* lived. One of the mice injected with dead *B. proteus* and lactic acid died after two days, the other two were living after 30 days' observation.

*Streptococci*.—0.5 c.c. of a 24 hour bouillon growth of streptococci and 0.5 c.c. of a 0.85 per cent lactic acid were injected subcutaneously into mice. There were 16 strains of hemolytic and 9 strains of viridans used, all were nonpathogenic for mice. All the animals remained alive after a three weeks' period of observation. Twenty-two mice had been injected with streptococci, in a previous experiment to test their pathogenicity, and were alive 8 to 14 days after receiving the inoculation, were injected subcutaneously with 0.5 c.c. of an 0.85 per cent lactic acid. All were living 10 days after the last injection.

#### DISCUSSION

The results obtained with the *B. subtilis* are negative throughout. Neither the addition of lactic acid to the culture medium, nor the injection of lactic acid into mice at the time of inoculation, cause the strain of *B. subtilis* used by us to become virulent for mice.

The results with the *B. proteus* are not so clear cut. Of the six control mice injected with 1 c.c. of a 24 hour old culture, one died in 24 hours; of the six mice injected with *B. proteus* and lactic acid, two died; of the six mice injected with *B. proteus* grown on 0.08 per cent lactic acid in bouillon, one died.

The lactic acid does not seem to have increased the virulency of the *B. proteus* for mice. *B. proteus* that have been killed by heating to 58° C. for one hour had the same effect as the living strain when injected with lactic acid. The results obtained in this work do not agree with those reported by Much, but confirm the observations made by Bachmann and also those of Lange and Yoskioka.

The injection of lactic acid into a mouse inoculated with an avirulent strain of streptococcus does not increase its virulency for this animal. The results were also negative if the lactic acid is injected several days after the streptococci.

The *B. proteus* was the only organism used by us that was toxic for mice. The apparent increased virulency of this bacterium for mice when combined with lactic acid can be more readily explained by its impairment of the defensive mechanism of the host, than by an actual change in the biological characteristics of the microorganism causing it to become more virulent.

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CONCERNING THE INFLUENCE OF THE INTRAVENOUS ADMINISTRATION OF MERCURY BENZOATE ON THE WASSERMANN REACTION OF APPARENTLY NORMAL INDIVIDUALS\*

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FOR many a generation, mercury had been the sheet-anchor in the treatment of syphilis. The discovery of chemotherapeutic methods, resulting in the introduction of arsphenamine and neoarsphenamine as specific remedies for the treatment of syphilis, have, for a time, caused the gross neglect of the mercurials. Following the subsidence of the arsphenamine wave of enthusiasm and the same appreciation of the value of organic arsenicals in the medical armamentarium, the value of the mercurials began again to be more and more appreciated.

The early use of mercury by mouth in the treatment of syphilis, a method still in vogue, to the inunction method, then, the intramuscular injection of mercury and finally the intravenous administration of this remedy, represents the various steps in the therapeutic refinements and the desire of exact medication.

The attempt on the part of some English and French syphilographers and to a minor degree, the efforts of a few American syphilographers to popularize intravenous mercurial therapy has caused us to study this problem.

In a previous communication, the author studied the influence of intravenous administration of arsphenamine and neoarsphenamine upon the Wassermann reaction of normal individuals. This study seemed to prove that these organic arsenicals were capable of influencing in a positive manner the Wassermann reaction of normal patients. The same results have also been obtained by Ravaut following similar lines of investigation.

It, therefore, occurred that it would be of value to determine whether or not mercurial preparations influenced the Wassermann reaction of apparently normal patients. With this object in view, twelve patients were selected, suffering from either one of the following skin conditions, particularly the chronic dermatoses, such as chronic eczema, lichen planus, sycosis vulgaris, etc. The treatments were given at weekly intervals and were administered intravenously.

The salt of mercury usually employed was the Benzoate and the dose varied from  $\frac{2}{5}$  of a grain to  $\frac{3}{5}$  of a grain. The salt of mercury was dissolved in normal salt solution. In a few instances, mercury cyanide was administered. A Wassermann reaction was performed on the serum of each patient before treatment was begun. Following the treatment, at various intervals, the complement fixing powers of their blood was tested.

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A study of the following table discloses that eleven patients had a negative Wassermann reaction before mercurial treatments were instituted. One patient had a 4+ reaction at the beginning of the treatment and in spite of twenty mercurial injections, the Wassermann still remained 4+.

The number of mercurial injections varied from eight up to thirty-seven. In nine of the patients, the Wassermann reaction remained negative as a result of the intravenous injections of the mercurials. In one instance, the Wassermann reaction became  $\pm$  after the twenty-second injection and then became negative when again taken after the twenty-ninth injection of the remedy.

In another instance, the Wassermann was negative before the mercurials were administered and became a 4+ after the fourth injection, remained positive 4+ after the twelfth injection and was still a 4+ after the eighteenth injection when the patient disappeared. This particular patient had an acute psoriasis of nine months' duration which involved the arms and legs.

#### RESUME

As a result of our study relative to the influence of the mercurials administered intravenously, upon the Wassermann reaction of apparently normal individuals, we can state:—

(1) It is our belief that the intravenous administration of the mercurials does not influence the Wassermann reaction.

(2) In our series as high as thirty-seven injections were given to a patient without producing a positive complement-fixation test.

(3) In one instance, where the Wassermann reaction was 4+ before treatment was instituted, the administration of twenty intravenous mercurial injections failed to influence the Wassermann reaction.

(4) In one instance, a patient suffering with psoriasis, developed a 4+ reaction after the fourth mercurial injection and this 4+ reaction persisted after eighteen injections of the remedy.

The effect which mercury intravenously administered seems to have upon the Wassermann reaction of apparently normal individuals, appears to differ from the effect which arsphenamine and neoarsphenamine, similarly administered, seem to exert upon the Wassermann reaction of similar individuals.

The hypothesis which we offer in explanation of our findings is as follows: It is generally admitted that the arsenicals have an affinity for the liver. This fact has been confirmed by Kolmer and Lueke in their study of the effect of the intravenous administration of the organic arsenicals, arsphenamine and neoarsphenamine upon rats and rabbits.

It is also generally believed that whatever the nature of the substance which causes a positive Wassermann may be, it is probably a substance of lipoidal nature. It would appear probable that the arsenicals in their action upon the liver cells may so alter their functions, true, probably only in a temporary fashion, as to cause either a change in the lipoids, or the lipoidal substance may flood the blood stream so that the serum, when examined at that particular time, produces a positive complement-fixation test (a positive Wassermann reaction).

On the other hand, it has been definitely established that the mercurials have a great affinity for the kidney structure. The mercurials do not profoundly influence the liver structure. As a result, the administration of the mercurials does not influence in a positive manner, the Wassermann reaction of normal individuals.

We advance this theory to explain the difference between the organic arsenicals and mercury in so far as it concerns the Wassermann reaction of apparently normal (nonsyphilitic) individuals.

CASE NO.	RESULT OF WAS- SERMANN BE- FORE TREAT- MENT	TREATMENT AT WEEKLY INTERVALS	RESULT OF WASSERMANN AFTER TREATMENT
1—W. H.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 3 inj. Wassermann negative “ 11 “ “ “ “ 18 “ “ ++ “ “ 29 “ “ “ “
2—J. A.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 1 inj. Wassermann negative “ 4 “ “ “ “ 12 “ “ “ “ 14 “ “ “ “ 19 “ “ “ “ 21 “ “ “ “ 23 “ “ “
3—W. H. W.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 7 inj. Wassermann negative “ 11 “ “ “ “ 19 “ “ “ “ 21 “ “ “ “ 23 “ “ “ “ 29 “ “ “ “ 33 “ “ “ “ 37 “ “ “
4—M. S.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 12 inj. Wassermann negative
5—P. S.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 4 inj. Wassermann negative “ 8 “ “ “ “ 15 “ “ “
6—M. S.	+ + + + +	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 18 inj. Wassermann + + + + + “ 19 “ “ “ + + + + + “ 20 “ “ “ + + + + +
7—J. E. W.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 3 inj. Wassermann negative “ 8 “ “ “
8—P.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	“ 11 “ “ “
9—S.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 19 inj. Wassermann negative “ 23 “ “ “ “ 27 “ “ “
10—I. G.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 4 inj. Wassermann negative “ 8 “ “ “ “ 15 “ “ “ “ 17 “ “ “ “ 18 “ “ “ “ 19 “ “ “
11—W. A.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 4 inj. Wassermann + + “ 12 “ “ “ + + + + + “ “ “ “ + +
12—I. L.	Negative	Hg. Benz. gr. 2/5 in 10 c.c. of N.S.S.	After 22 inj. Wassermann negative

# DIAGNOSTIC VALUE OF THE KAHN TEST FOR SYPHILIS\*

## A PRELIMINARY REPORT

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MUCH interest has been developed in recent years in precipitation tests for syphilis, this interest being undoubtedly due to the fact that physicians have for a long time felt the need of a laboratory test for syphilis less complicated than the Wassermann. The numerous sources of error inherent in the Wassermann test have to this day kept many physicians from depending on this test. The fact that two different laboratories are likely to give two different Wassermann results on one specimen of blood is well known. Physicians, not only in the United States, but in other countries, are therefore showing an unusual interest in simple precipitation tests with a hope that they might obtain a more correct diagnosis as well as a better check on treatment in syphilis. In Germany the Meinicke and Sachs-Georgi reactions are being discussed to a considerable degree at the present time. In England the Dreyer and Ward Sigma reaction is receiving some attention. In this country, however, the Kahn reaction is finding particular favor.

I shall not go into detail regarding the Meinicke, Sachs-Georgi and Dreyer and Ward reactions, except to say that the Meinicke and Sachs-Georgi reactions have not been accepted with favor in this country or in England. One of the main reasons for this possibly lies in the long incubation period of forty-eight hours which is required to bring out the precipitates in these reactions. This incubation frequently causes bacterial contamination, making the final reading of results quite difficult.

Several workers have reported on the Sachs-Georgi reaction in this country. Kilduffe,<sup>1</sup> who has made a special study of the diagnostic value of this test, sums up with the following significant statements:

1. The reaction is neither as delicate nor as trustworthy as the Wassermann test.

2. A diagnosis of syphilis or conclusions as to the results of treatment, cannot be based upon the results of a Sachs-Georgi reaction with safety, and the reaction does not seem suitable for general use for this purpose.

Craig and Williams<sup>2</sup> also find this test undependable for the serum diagnosis of syphilis.

Regarding the Dreyer and Ward reaction,<sup>3</sup> it is generally believed to be no less complicated than the Wassermann test itself. The antigen has to be prepared with unusual care, and from nine to twelve tubes must be set up for each test.

\*From the Duemling Clinic.

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Turning to the precipitation test as proposed by Kahn<sup>4</sup> we find an altogether different condition. The test is far less complicated than the other precipitation tests. In the case of the four-plus and three-plus reactions, furthermore, the precipitates are so marked that they can be seen without the slightest difficulty. This in contradistinction to the other precipitation tests which require an agglutinoscope to read the results. The two-plus and one-plus reactions in the Kahn test can also be seen with the naked eye after some little experience. A particularly valuable feature of this test is the so-called spontaneous reaction. After mixing the proper amounts of serum and antigen, one will frequently observe in the case of strongly positive serums a definite precipitate within a few minutes. This should be of help in special cases of blood transfusion when it is desired to eliminate active syphilis in the donor. It should also prove to be of use in emergency surgical operations when it may be necessary to know the existence of syphilis. About 90 per cent of the stronger reactions show marked precipitation in the Kahn test after three to four hours incubation. The test as a whole, however, is incubated overnight in order to bring out the weaker reactions.

A brief summary of the main points of the Kahn reaction is given by Keim and Wile:<sup>5</sup>

1. The patient's serum is employed in undiluted form, it having been shown by Kahn that diluting the serum with salt solution delays the rapidity of the reaction.

2. The antigen is prepared so as to contain a high content of antigenic material. This is obtained by using an alcoholic extract of dried heart muscle, which has been previously extracted with ether, thus removing fat and other nonspecific substances.

3. The antigen is diluted for the test with approximately the smallest amount of physiologic sodium chloride solution which will hold it in solution, rendering it thereby susceptible to precipitation when mixed with positive serum.

4. The antigen and serum are employed in that relationship which will, in the author's opinion, result in maximum precipitation.

We became particularly interested in the Kahn test after reading Keim and Wile's unusually favorable report on this test. These workers made an extensive study of the test at the Dermatological Clinic of the University of Michigan Hospital at Ann Arbor. Altogether they studied about three hundred fifty cases of which one hundred ninety-three were patients in various stages of syphilis. In their work each Kahn test was checked with two Wassermann tests in two different laboratories. In their cases of primary syphilis all the serums which were positive with the Wassermann reaction were positive also with the Kahn; many weak Wassermann positives proved strong positives with the Kahn; and one with a negative Wassermann gave a positive Kahn. In the cases of secondary syphilis there was the same sensitiveness noted in the Kahn reaction as compared with the Wassermann test. Their study of the cases of tertiary syphilis led them to conclude that the Kahn reaction is less sensitive in those cases than the Wassermann with prolonged



ice-box fixation, but more sensitive than the Wassermann with short ice-box fixation. In cerebrospinal syphilis the results are practically the same with both tests. In congenital syphilis the Kahn reaction gives rather more positives than even the Wassermann with long ice-box fixation, and in latent syphilis the sensitiveness is decidedly in favor of the Kahn reaction. In their series of one hundred fifty-seven nonsyphilitic cases, which consisted of various forms of dermatosis, all were negative to the Wassermann test, one hundred fifty-four were negative to the precipitation test, one was a case of acne giving a two-plus Kahn reaction, and one case of smallpox and one of diabetes, both giving a one-plus.

#### EXPERIMENTAL PROCEDURE

In this laboratory we have been running the Kahn precipitation test in conjunction with the Wassermann test on all serums and spinal fluids sent us, and now have one hundred seventy-five cases on which we can report findings.

In making our Wassermann test we use the human cell system, and two antigens, a plain alcoholic extract and Noguchi antigen. The latter, in our experience, is as sensitive as a cholesterinized antigen without the tendency to give false positives. Water-bath incubation is used.

In performing the Kahn test the author's<sup>4</sup> routine was rigidly adhered to. The serums were inactivated for thirty minutes at 56° C. Two cholesterinized antigens were used. These were diluted by adding three volumes of normal salt solution to one of antigen. To 0.3 c.c. quantities of each serum was added 0.05 c.c. of antigen; the tubes shaken for three minutes, and examined for spontaneous precipitation which might then be observed in all strongly positive serums. The tubes were then placed in the incubator for from twelve to eighteen hours, and the final readings made according to the following scheme, as designated by Kahn: A precipitate consisting of one or several large clumps, four-plus; a large flocculent precipitate, three-plus; moderate sized flocculi or granules, two-plus; small sized flocculi or granules, one-plus; fine flocculi or granules, plus-minus; no precipitate, negative.

TABLE I

	WASSERMANN	KAHN REACTION	
		SPONTANEOUS	FINAL
153 serums	-	No	-
10 "	++++	Yes	++++
1 serum	+++ to ++++	Yes	++++
1 "	+++	Yes	+++

An important point in the preparation of the serums is that they shall be absolutely clear, otherwise the reading of the weaker reactions is not easy. Slightly opalescent serums present no particular difficulty. Anticomplementary serums, if clear, are entirely suitable for the Kahn test, since this reaction is positive or negative according to whether or not the specific reagin is present. This is an obvious advantage over the Wassermann reaction, as anti-complementary serums are not infrequently encountered in which a reading cannot be accurately made.

The accompanying table shows that in one hundred sixty-five of our cases the Kahn test checked exactly with the Wassermann.

As shown in Table II, in five cases the Kahn reaction proved more sensitive than the Wassermann. One, a treated case with three negative Wassermans in the last two years, gave a positive Kahn at the time of the last negative Wassermann. Another case diagnosed by the physician as aortic aneurism with psychosis showed a negative Wassermann but a two-plus Kahn. In a case of carcinoma of the rectum we obtained a negative Wassermann and a two-plus Kahn. (This serum was also sent to Doctor Kahn for examination, who reported a doubtful Wassermann and a two-plus Kahn.) In an undiagnosed case the routine Wassermann was negative, Kahn two-plus. (Doctor Kahn's findings in this case also were the same as ours.) A treated syphilitic from the Feeble-Minded School gave a negative Wassermann and a two-plus Kahn.

TABLE II

	WASSERMANN	KAHN REACTION	
		SPONTANEOUS	FINAL
1. Treated case; three negative Wassermans in past two years .....	-	Yes	+++
2. Diagnosed as aortic aneurism with a psychosis ....	-	No	++
3. Carcinoma of rectum, syphilis questioned .....	±	No	++
4. Undiagnosed case .....	-	No	++
5. Treated case from School for Feeble-Minded .....	-	No	++

The five spinal fluids of Table III which were submitted to this test gave rather unsatisfactory results. One, a case of epilepsy, was negative with both the Wassermann and the Kahn, as also an undiagnosed case, and a case of spastic paraplegia. A case of tabes dorsalis with a negative Wassermann, and one of general paresis, both showed merely a milkiness of the spinal fluid-antigen mixture.

TABLE III

	RESULTS WITH SPINAL FLUIDS		
	WASSERMANN	KAHN REACTION	
		SPONTANEOUS	FINAL
1. Case of epilepsy .....	-	No	-
2. Undiagnosed case .....	-	No	-
3. Tabes dorsalis .....	-	Milky	Milky
4. Spastic paraplegia .....	-	No	-
5. General paresis .....	++++	Milky	Milky

It must be remembered in this connection that according to Kahn, the application of this test to spinal fluids is still in the experimental stage.

No cases of primary syphilis are included in our series, but all the syphilitics were in the later stages of the disease. The cases on which the tests were made simply as a matter of routine and found to be negative to both the Wassermann and the Kahn, include the following diseases: furunculosis, arthritis, asthma, spastic paraplegia, secondary anemia, spondylitis, neurosis, myocarditis, cholecystitis, hyperthyroidism, epilepsy, eczema, neu-

ritis, hypertension, brain tumor, sciatica, varicose ulcer, urticaria, and glandular tuberculosis.

#### CONCLUSIONS

1. The Kahn reaction is a simple and practical test the technic of which is easily mastered, the reading of the results requiring comparatively little experience, except in distinguishing between the weaker reactions.

2. In the application of the Kahn reaction there is less chance of error in technic than in the Wassermann since the only elements entering into its performance are antigen and patient's serum.

3. As at present developed the test does not seem of particular value in the case of spinal fluids.

4. Our results indicate that the Kahn precipitation test is a most valuable check on the Wassermann test and should be done on all serums submitted for the Wassermann.

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# LABORATORY METHODS

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## SOME OBSERVATIONS ON THE LARGE MONONUCLEAR INDEX IN CHRONIC APPENDICITIS\*

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IN the routine blood counts on suspected cases of appendiceal infections both chronic and acute, it was noticed that the large mononuclear index in the differential count was rather irregular, often showing a definite increase. As a mononuclear increase is expected only in such conditions as malaria, typhoid, etc., the following studies were undertaken to establish the frequency of such occurrences in appendiceal involvements which might prove to be a valuable diagnostic aid.

While this study was in progress my attention was called to Friedman's<sup>1</sup> article detailing the same kind of study, in which he established the fact that in 87 per cent of chronic appendicitis cases the large mononuclear index was greater than 5 per cent, which he took to be normal. Friedman gave figures for both the large mononuclears and transitionals, but grouped them in one total for his final results.

It has been my classification for some time to regard the mononuclears and transitionals in one group. Pappenheim as quoted by Naegeli<sup>2</sup> points out that the change from transitional to polymorphonuclear neutrophile, in all probability, never occurs. However the neutrophiles, eosinophiles, and basophiles arise from the same embryonic cells (myeloblast) as do the mononuclears and transitionals, and it can easily be conjectured that certain stimuli would cause an increase in mononuclear formation instead of neutrophile formation.

Indeed it is just this, presumably, that occurs in malaria, and typhoid and it seems probable that the same result occurs in certain other types of infections. In Friedman's series of cases the diagnosis of chronic appendicitis is well established in each case, and it seems certain that in this condition the large mononuclears are definitely increased. He states, however, that the increase in large mononuclears is diagnostic of this condition, of course with the proper physical findings concurring. It occurred to me, however, that the mononuclear increase might not so constantly point to lesions of the appendix, and that if such a chronic infection gives an increase of mononuclears it might be possible for the same result to occur in chronic infections of other regions. Friedman points out a number of conditions that give no increase in mononuclears, but his conclusions in this respect are based on rather meager evidence.

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To throw light on these points, and to establish the value of the differential as a diagnostic guide in chronic appendix, a study of several series of cases was made by compiling data from the records after discharge. Obviously none of the work was done under experimental conditions, and perhaps some of the value of these observations is lost thereby. All the blood counts however were done by myself, and all the pathologic examination of specimens was done by Dr. N. E. Leake, thus eliminating the varying personal element in those respects. In each case the diagnosis of the condition was arrived at by the surgeon without being aware that such a study was being made. In most cases the differential was made on 100 cells, in a few on two hundred. Of course I am aware of the attending percentage of inaccuracy in such counts; but, due to the low total count, it is very seldom expedient in routine work to include more than 100 cells in these counts.

The first study was of a series of 52 cases with a preoperative diagnosis of chronic appendicitis. Of these, 40 showed chronic changes, or acute exacerbations of a chronic appendix (two cases). Of these 40 cases 19 (47.5 per cent) showed a large mononuclear count greater than 5 per cent.

This variation from Friedman's figures is rather wide and it was thought, because of the fact that the preoperative diagnosis was not arrived at under experimental conditions, thus insuring exhaustive evidence, that some of the cases could possibly be excluded. Chronic changes were found in the specimen of all cases. But according to Gaylord and Aschoff<sup>3</sup> some of these apparently chronic changes might be physiologic. Excluding cases in which the pathology was of such a nature (distal end fibrosis) that it might be physiologic the figures rearrange themselves slightly. Six such cases are encountered in the series. None of these show increased mononuclears. This leaves us 34 cases of absolute chronic appendicitis with 19, or 55.9 per cent, showing a mononuclear increase. This still leaves a wide discrepancy as compared to Friedman's figures.

It is interesting to note that 5 of the original 52 cases revealed normal appendices on examination, and that 1 of these has an increase to 9 per cent large mononuclears. Four of the 52 were found to be acute upon pathologic examination of which 1 showed a count of 6 per cent mononuclears.

Thinking that if a mononuclear increase was to be taken as evidence of an appendiceal lesion, acute cases might show an increase in these cells. A study of cases was undertaken with this in view.

The second series was of 77 cases with a preoperative diagnosis of acute appendicitis. Of these 77 cases 67 proved, on pathologic examination, to be actually acute cases. Of these 67 cases 9, or 15 per cent have large mononuclear increase over 5 per cent. Four of these 77 cases proved to be chronic, 2 of them having mononuclear counts over 5 per cent. Three of the 77 proved to be normal, none having increased mononuclear counts.

It is readily seen from these cases that the mononuclear increase is not due to the appendix being infected, but rather, it is inferred, to the type of infection; as only 15 per cent of acute cases have a mononuclear increase. It is possible that the 15 per cent represent cases of long standing, the acute features

of which masked the chronic changes on examination. It seems likely from these findings, that the mononuclear increase was due to a mild stimulation by a chronic infection. To determine whether or not this increase was due to the seat of infection, a third series was studied.

In this series, 15 cases of chronic cholecystitis, uncomplicated, are contained. Seven, or 46.6 per cent of these show an increase in mononuclears. Only one out of five acute cases contained in the series shows a mononuclear increase. Two cases, which were complicated by chronic appendicitis, showed an increase of mononuclear cells.

These findings are approximately the same as found for chronic appendicitis and although based on a small number of cases certainly point to a source of future study. They also warn the diagnostician to not hastily accept a large mononuclear increase as a sign of chronic appendicitis only. A fourth series of cases was studied to see if more information could be gained on this phase of the subject.

This series contained 23 uncomplicated cases of chronic salpingitis. Of these 7 or 30.5 per cent, showed an increased mononuclear count. Two cases complicated with chronic appendices also have an increased mononuclear count.

Here again we find a chronic condition giving some increase in the mononuclears; and we are forced to conclude that the appendiceal lesion is not alone responsible for a mononuclear increase.

#### SUMMARY

A series of cases of chronic appendicitis were studied to determine the value of an increased mononuclear count as a diagnostic aid.

It was found that 47.5 per cent, or perhaps 55.9, excluding possible physiologic appendicitis, of the cases actually have an increase in large mononuclears. This is at wide variance with Friedman's findings. To establish whether or not any lesion of the appendix was capable of increasing the mononuclears, a study of acute cases was done showing that only 15 per cent showed a mononuclear increase.

To determine the possibility of a mononuclear increase being selective for a chronic infection of any site, rather than of the appendix only, two series were studied—(one of salpingitis and one of cholecystitis) revealing that 30.5 per cent and 46.6 per cent respectively of these cases showed a mononuclear increase over 5 per cent.

In these series the large lymphocytes were studied. In series 3 (cholecystitis) it was found that 10 or 58.8 per cent of the 17 chronic cases show high large lymphocyte counts, and this suggested the possibility of a significance of some kind. However none was established in the other conditions as the lymphocyte count was too variable.

#### CONCLUSIONS

1. In about 50 per cent of the cases of chronic appendicitis an increased mononuclear count is an aid in diagnosis.

2. An increase in mononuclears is in all probability not due to the appendix being infected but to the character of the infection (chronic).

3. In chronic salpingitis and in chronic cholecystitis a number of cases (30.5 per cent and 47.5 per cent) showed a mononuclear increase.

4. The diagnostician should not rely too heavily in doubtful cases on the findings of increased mononuclears as a sign of chronic appendicitis, but should bear in mind the possibility of at least two other conditions giving the same blood picture.

It is to be borne in mind that this study was done under routine conditions and therefore is not to be taken as an experimental study but rather a survey of routine results.

Further studies are being done, on an experimental basis, to more securely establish the value of the above observations.

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## THE EMPLOYMENT OF VEGETABLE EXTRACTS IN THE WASSERMANN REACTION\*

BY EMIL WEISS, M.D., CHICAGO, ILL.

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THE antigens used in the Wassermann reaction have been obtained, as a rule, from animal origin. The historical review of this subject has been covered in detail by several authors. We wish only to mention those publications that have a direct bearing on our problem.

Tribondeau<sup>1</sup> extracted material from vegetable origin and obtained reliable antigens as compared with those from animal origin. This author used peas, beans and corn. These were ground up in a mortar, washed with absolute alcohol, acetone or ether, then centrifuged, the supernating fluid was decanted off and the sediment resuspended; this process was repeated until the supernating fluid was clear. The vegetable was then dried and extracted with alcohol or acetone and concentrated by evaporation. This author found the antigens from peas gave the most consistent results. Heinlein<sup>2</sup> extracted an antigen from potatoes. The peeled and grated potatoes were treated with five times their volume of alcohol. From this step on the technic was the same as usually employed in the preparation of organ extracts. Heinlein found that the potato antigen only gave positive results with some of the syphilitic sera. This author also found that the addition of cholesterine did not give a larger percentage of positives as compared with the same procedure with his control animal extracts. This potato antigen deteriorated quickly, sometimes after two weeks standing it was not usable.

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The writer thought it worth while to prepare extracts from a series of vegetables and carefully test these extracts as to their usefulness as antigens for the Wassermann reaction.

The majority of serologists at the present time use several different antigens in the technic of the Wassermann reaction. It would be advantageous to be able to use, in a confirmatory way, antigens from vegetable as well as from animal origin. In addition to the vegetables being useful for preparation of the extracts, they are very easily obtained. The procedure of the extraction is very simple.

#### TECHNIC OF PREPARATION OF ANTIGENS

Antigens were prepared from the seeds of the following vegetables, viz: ricinus, poppy, rape, hemp, flax, squash, watermelon, sunflower, radish, mustard, beans, barley, corn, peas, lentils, and from the meat of walnut, cocoanut and olive.

All foreign substance was removed and the material was then ground up into very small particles and dried, then two volumes of alcohol or acetone added and placed in a shaking apparatus for twenty-four hours. After this, the flasks were set aside at room temperature for five or six days. If a shaking apparatus is not at one's disposal, it can be kept for ten or twelve days at room temperature, but must be shaken up several times a day. The contents of the flasks are then filtered through a double layer of hard filter paper, the clear extract was transferred to amber colored bottles and kept at room temperature. It is better to keep these antigens at room temperature than to keep them in the ice box at a lower temperature. If the extracts are cooled to a lower temperature after they are prepared, weak extracts have a tendency to become cloudy and stronger extracts show sediment. It is possible to clear up the cloudiness and redissolve the sediment by gently heating the flask. This can be eliminated if the ice box is not used to preserve the antigens.

#### THE EXAMINATION OF EXTRACTS

Kaup<sup>3</sup> gave a very accurate method for the titration of the proper amount of antigen to be used. This author determined the different zones of each antigen in the following way:

- (1) The extract, amboceptor and sheep cells suspension.
- (2) Extract, normal serum, amboceptor and sheep cells suspension.
- (3) Extract, complement, amboceptor and sheep cells suspension.
- (4) Extract, normal serum, complement, amboceptor and sheep cells.
- (5) Extract, syphilitic serum, complement, amboceptor and sheep cells suspension, varying the amount of antigens from .5 c.c. to .0001 c.c.

According to Kaup's method, with a useful extract one distinguishes from the largest down to the smallest doses, the following reactions: A large amount causes a precipitation of albumin, (precipitation zone), a slightly smaller amount causes hemolysis, (hemolytic zone), and in still smaller doses shows inhibitions, (inhibitions or anticomplementary zone), a smaller amount shows





the specific reaction (specific or antigenic zone) and the smallest dose shows hemolysis, (or inefficient zone). This method, with minor modifications, was used for the examination of the vegetable extracts. The vegetable extracts, examined in this manner, gave the same reactions in the various zones as was found in different organ extracts.

In order to show the influence that the extracted substances alone have on the different zones, we evaporated carefully an alcoholic olive extract at low temperature to one-fourth of its original volume, this was then made up to volume with normal salt solution. This extract showed the same reaction in the different zones as the original extract, the only difference being that a slightly larger quantity had to be used.

1 c.c. of a 1 per cent solution of cholesterine was added to 10 c.c. of an alcoholic olive extract. This cholesterinized vegetable antigen was made stronger in the same way as an animal extract.

#### CONCLUSION

The vegetable extracts examined by us as to their usefulness as antigens in the Wassermann reaction, can be divided into three groups: Group 1, olive and cocoanut. The green olives are far better than the ripe ones. The extracts from these are as useful as the extracts from animal tissues. The antigenic or specific zones are clear cut and reliable. The only difference in the alcoholic and acetone extracts is that with the latter the zones are shifted a little upwards, that is, that same reaction but a little larger amount of the extract is required to parallel the alcoholic extracts. The differences in the results between the normal animal extracts and these two vegetable extracts were never greater than one always finds between two different animal extracts. The vegetable extracts never showed a negative reaction where an organ extract showed a complete inhibition, and the vegetable extracts never give strong inhibition when the organic extracts show complete hemolysis. (Table I.)

Group II. Ricinus seed, flax seed, hemp seed, radish seed, sunflower, poppy seed, rape seed, peas and mustard seed. With these extracts the antigenic or specific zone is very narrow, the difference between positive and negative sera is not great enough to be used as a reliable antigen in the Wassermann reaction.

Group III. Corn, walnut, beans, barley, watermelon seed, squash seed and lentils. The extracts of this group of vegetables are absolutely useless as antigens for the Wassermann reaction. There is no difference between positive and negative sera, the specific or antigenic zone does not exist.

#### SUMMARY

The alcohol or acetone extracts of olive and cocoanut are very useful as antigens for the Wassermann reaction. The difference in the results between vegetable and animal extracts is no greater than that between two different animal tissue extracts. The addition of cholesterine increases the breadth of the specific zone the same as with animal extracts. For the extraction of

vegetables, alcohol, 95 per cent or absolute and acetone, are equally useful. The durability of the vegetable extracts at room temperature is the same as that of the animal tissue extracts.

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### WASSERMANN SYSTEMS\*

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THE technic of the original Wassermann test did not concern itself with the problem of systems; it demanded sheep's corpuscles and it was not until some time later, when various vulnerable points in the test were beginning to manifest themselves, that it occurred that one of the weak points might be in the hemolytic system; it was not long after this suspicion arose until every imaginable kind of corpuscle had its supporters as being the ideal one for complement-fixation work, and this usually without defining the particular weakness of the one or the superior points of the other.

What criteria shall be accepted as indicating the system of choice? First and foremost, I should say that system is best for which the most potent amboceptor can be prepared; secondly and of almost equal importance in my opinion, is the selection of a system for which the particular serum to be tested contains the least amount of lysin; thirdly, we must consider the availability of corpuscles; fourthly, we must consider the possibility of more or less iso-hemolysin and fifth and of rather minor importance is the question of clarity of contents of our tubes.

This paper is based upon the investigation of those animals which would enable us to meet the requirements of the third criterion—those bloods most available, namely, sheep, beef, human, chicken, duck, goose, guinea pig, and rabbit.

One of the earliest and most popular deviations from the original Wassermann technic was the employment of the human blood system as popularized by Noguchi; this system has among its recommendations the fact that it is sponsored by this brilliant worker; also in its favor is the fact that nothing is cheaper or more available than human blood; the use of amboceptor papers in this system is said to be an advantage. Weighed against these advantages is the fact that an antihuman amboceptor of high titer cannot be produced and the writer feels that rapidity and clearness of action in the second incubation

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tion permits of the exercise of a great deal of judgment in interpreting readings. Wm. C. Williams<sup>1</sup> has shown the dangers of error when the human system is used, resulting from the presence of antihuman hemolysin in guinea pig serum, specific for corpuscles of groups one and two. The same author maintains that iso-hemolysins for human cells exist in human serum, necessitating the typing of corpuscles used in the test. Such sources of error would tend toward false negatives; of even graver importance is the fact that, using the human system, a considerable number of false positives are encountered. Some years ago, before I had investigated the adaptability of the various systems, while the slaughter houses were idle as the result of the prevalence of foot and mouth disease, I was glad to avail myself of the antihuman system in spite of its faults. Today idle stock yards would cause me but little concern.

Of the different fowls used in complement-fixation work, my experience is limited to the use of the blood of the chicken, duck, goose, and guinea fowl; these all are fairly adaptable under the requirements relative to the potency of artificial amboceptor, but the great majority of human sera are rich in natural amboceptor hemolytic for these corpuscles and in this respect they offer no advantage over the sheep system; also militating against their use is the fact that it is very difficult to wash the corpuscles of fowls to the point where they give a perfectly clear picture in negative tubes; there seems to be something in nucleated corpuscles which gives more or less of a murky fluid after hemolysis of corpuscles which is sometimes very annoying in reading end results. In their favor, of course, is their usual availability. It is very comforting to know that systems of such utility, possessing no greater disadvantages of a serious nature, are always at hand if an emergency should arise.

Grouping the sheep, ox, hog, and guinea pig, they all meet requirements in about equal measure, producing amboceptor in the rabbit of fairly high titer; against them in almost equal measure, I have found that human serum contains great amounts of natural amboceptor, being greatest in antiguinea pig and lowest in antiox, but the difference is so small as to be of no practical consequence; however, individual sera vary considerably against individual corpuscles; one serum may be rich in antisheep amboceptor and poor in antiox or antiguinea pig, and in doubtful cases we select our system accordingly; our greatest reliance is placed in sheep, ox, and guinea pig, the latter, because, like the poor, it is always with us; we have one amboceptor specific for three systems; we are very partial to the rabbit also, but for obvious reasons, our one amboceptor has not been made to include this animal.

While this paper has to do only with systems, the proper pursuit of the subject must of necessity lead into the by-path of some discussion of methods of complement fixation. That a biological test be of most practical utility, it is necessary that it be as free from error as possible, but since all such tests are more or less subject to error, it becomes essential that all such sources be known; that provision may be made for their recognition and interpretation and when such errors are inherent and cannot be remedied, it becomes necessary to adopt a substitute test or a modification which is free from such error.

The causes of Wassermann error inherent in the test itself are 1, presence



of but few antibodies, this resulting from (a) heating of serum; (b) effect of treatment; (c) early stage or so-called latency of the disease. 2, presence of natural antish sheep amboceptor in quantities sufficient to destroy the proper ratio of Wassermann factors. Noguchi<sup>2</sup> has shown that during the first five minutes of inactivation about 49 per cent of luetic antibodies are destroyed; during the next five minutes 20 per cent disappear and at the end of thirty minutes inactivation but 25 per cent remain. Thus the necessity for conserving antibodies, especially in intensively treated and in early and latent cases, becomes apparent; and as these form a great part of the cases coming to us for diagnosis, it is equally apparent that a large number of these cases do not readily lend themselves to the unmodified Wassermann technic, limiting itself as it does, to the antish sheep system. The second great source of error is the presence of antish sheep amboceptor in quantity sufficient to destroy the proper ratio of Wassermann factors. Excess of amboceptor is disconcerting only when antibodies are few. It is my experience that a serum containing enough natural antish sheep amboceptor and luetic antibodies to cause a four-plus reaction, shows no hemolysis when considerable amboceptor is added, but frequently does show effect in less strongly positive cases; it is the loss of balance between amboceptor, in excess and antibodies deficient in amount, that causes negative reactions where positive ones should obtain; the smaller the amount of antibodies, the more probable that excess of amboceptor will destroy a delicate balance. Where antibodies exist in great numbers a comparatively rough approximation of reagent quantities may be tolerated, but in the presence of small amounts a most delicate manipulation is imperative. Most human blood contains enough amboceptor, either antish sheep, ox, hog, rabbit, guinea pig, chicken, duck, goose or guinea fowl, one, more or all of these, to cause hemolysis in very considerable amount. I have frequently seen sera capable of causing hemolysis in proportion of .1 c.c. serum to 1.5 c.c. of corpuscles in 5 per cent suspension. The complement of human serum, while usually not in great amount, is nevertheless quite constantly present and increases the effect of the natural amboceptor. The Wassermann technic, while demanding careful titration of amboceptor and complement added to the test, takes no cognizance of the amount of these factors naturally present in the serum; it does not seem reasonable that a serum containing a great amount of amboceptor, should have added to it the same amount of this ingredient demanded by a serum containing little or none of this body. It is obvious that each serum to be examined should be titrated as carefully as any other ingredient of the test; each serum should be individualized; it should be carefully titrated for its specific amboceptor and complement content; the presence of anticomplementary substances should be sought, and a comparative estimation of antibodies should be made.

Of all complement-fixation tests, the writer considers the Hecht-Gradwohl test the most widely applicable and useful; this method is useful not only as a complement-fixation test, but because of the amount of knowledge it yields concerning the peculiarities of each serum; it indicates antibodies; it shows the presence of anticomplementary substances and the kind of amboceptor, and complement present; armed with this knowledge, the serologist is in position

to properly interpret the significance of the various tests, and to select the one best suited to the serum to be tested.

The Hecht-Gradwohl test makes use of the natural antishoop or other amboceptor and natural complement and is performed with unheated serum; it is therefore designed to overcome the great sources of error encountered in the original Wassermann test.

The technic as worked out by R. B. H. Gradwohl of St. Louis is as follows: 14 tubes are placed in a rack; into each tube is placed .1 c.c. of the patient's serum; then into the first ten tubes is placed descending amounts of normal salt solution; in the first tube .9 c.c. descending to .1 c.c.; then in these ten tubes are placed ascending amounts of sheep's corpuscles, beginning with .1 c.c., and ending with 1 c.c.; this gives the first ten tubes equal volume. These ten tubes are for the purpose of obtaining the hemolytic index—the hemolyzing power of the serum. In the next three tubes are placed graded amounts of acetone insoluble antigen, .2 c.c.—.15 c.c. and .1 c.c. this diluted to one-half the Wassermann strength. The last tube contains only serum and is the control tube. These last four are then brought to equal volume with salt solution; the rack is then placed in the water-bath at 37° for one-half hour, and frequently shaken. That tube which shows complete hemolysis of the greatest amount of sheep's corpuscles is then noted and gives the hemolytic index. Sheep's corpuscles are then added to the last four tubes according to this index; if this is from 1 to 4, one-tenth c.c. of corpuscles is added; if from 5 to 7, fifteen one hundredths and if from 8 to 10, two-tenths c.c. The rack is then placed in the water-bath and results read as in the Wassermann test, when the control tube shows complete hemolysis.

It will be noted that the above technic calls for the antishoop system; antishoop amboceptor is rather frequently absent from human serum; for such contingency we keep in the ice box corpuscles of ox, rabbit, guinea pig and the several fowls and it is the work of but a few minutes to find which system lends itself to the balking serum. I remember no case where at least one system could not be applied except in infants and in those rare instances where failure of hemolysis is due to the absence of natural complement; in such cases this is supplied and the test proceeds. In our routine work we run all sera, using the Hecht-Gradwohl and Wassermann tests, using sheep system of corpuscles. If we find no antishoop amboceptor present, or if in only small amount, we know that is the ideal system for the Wassermann test as one great source of error does not exist; we then substitute that system for the Hecht-Gradwohl test for which the greatest amount of amboceptor is present and proceed as with the Gradwohl technic. We rarely need resort to fowl blood.

If the Hecht-Gradwohl test is positive and the Wassermann negative (the reverse can never be true) we consult the hemolytic index; if this is high we repeat the Wassermann, using a system for which the serum shows a low amboceptor content, employing both heated and unheated serum.

These two tests, using only the antishoop system, disagree in about 10 per cent of all cases and in most cases, at some stage of treatment; by manipulating

the systems as just described, they are made to practically approximate in results, except in treated cases.

#### CONCLUSIONS

1. For complement-fixation work there is no one universally adaptable system. The amboceptor content of the serum to be examined determines the system most suited.

2. That system for which the serum to be tested contains the least amount of natural amboceptor is best suited to the Wassermann test.

3. Conversely, that system for which the greatest amount of amboceptor exists in the serum to be tested is best suited to the Hecht-Gradwohl test.

4. Most human sera contain a great variety of amboceptors in varying amounts.

5. Most sera contain a considerable amount of some one amboceptor, and are poor in a certain one, indicating the system best suited to it for Wassermann and Hecht-Gradwohl tests.

6. One specimen of rabbit serum may yield amboceptors specific for several systems.

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## EDITORIALS

### *Uremia*

THE daily clinical use of the term uremia has produced an easy familiarity and we are inclined to believe that in using it, we refer to a process well understood by all. On the contrary, the farther the study of uremia progresses, the more convinced we must become that we actually know amazingly little of the pathological condition. When the term was first adopted, the impression was, as the name implies, that the symptom complex arises from non-excretion of the normal urinary substances, and the retention particularly of urea in the blood. Hewlett fed himself urea, producing an unusually high urea concentration in the blood, without at the same time establishing any of the clinical symptoms of uremia. This, and the work of many others, established the error of the earlier conceptions. Today we still visualize the disease as secondary to and the result of severe kidney disease.

The most recent interpretation is that uremia is not due to primary renal damage, but is a general metabolic disturbance with resulting damage to many organs and tissues, among which there happens to be the kidney. In acute nephritis with edema, resulting from infection or other cause, we have usually understood a primary renal pathology. But here a change has oc-



curred which affects most of the cells of the body, presumably accompanied by alterations of the surface tension of fluids and of the permeability of cells. The renal epithelium is affected, but to no greater degree than the other tissues. As Marriott points out, these patients are not edematous because the kidneys fail to excrete water, but because the tissues hold the water and deliver little to the kidneys for excretion. This form of so-called nephritis follows damage by cellular poisons, which are often of the nature of bacterial toxins. With the removal of such poisons recovery is often complete, and the residual damage is no greater in the kidneys than in other body tissues. The frequency of acute nephritis following accessory sinus infection with complete recovery after removal of the original source of poisoning is but an example.

Interstitial nephritis is a true inflammatory disease of the kidney with resulting fibrous changes, but other forms, such as the acute nephritis just mentioned, are often systemic diseases, in which the part played by the kidney is incidental to the wider picture. According to this conception, uremia does not come from retention of poisonous or non-poisonous excretory products, but from general tissue damage by a specific metabolic poison. This poison presumably affects the kidneys at the same time that it is causing damage elsewhere.

Riesman remarks, "Of the important condition we call uremia so little is actually known that it constitutes an opprobrium of clinical medicine. Like the cause of cancer it is an unsolved riddle. We can add almost nothing today to the definition formulated many years ago by Bouchard: uremia is caused by a complex poisoning in which share, in unequal proportions, all the poisons introduced into the system or manufactured by it." Hamman defines uremia as the term used to designate a group of intoxication symptoms associated with renal insufficiency. This is as concise and as complete a definition as can be advanced in the present state of our knowledge.

It is true that uremia is the end result of nephritis but not all cases of nephritis end in uremia. Nephritis, as we understand the term, includes quite a variety of clinical entities and even in individual members of this general group, death may occur from a variety of causes.

Riesman points out that although the great multiplicity of symptoms may be thought to argue against a unitary cause for uremia, there are good reasons for the belief in a single primary poison. Whatever the nature of this poison or uremotoxin, whether it be a toxalbumin or a nonprotein poison, whether it be a crystallizable substance such as suggested a few years ago by Foster, we do know that it has a chemical affinity for many tissues and organs. This tropism is easiest recognized in its action on the liver, the central nervous system, the cardiovascular system including particularly the capillaries, the neuromotor mechanism, the adrenals and the kidneys themselves. Whatever the nature of the poison, it interferes with the normal functioning of these various structures. The multiplicity of lesions and the varying susceptibility of individuals explain in part the multiplicity of symptoms recognized in cases of true uremia.

Riesman classifies these symptoms under several headings, as follows: nervous manifestations, including aphasia, monoplegia, hemiplegia, headache, disturbances of vision and of hearing, neuralgia, convulsions and coma; mental or psychologic manifestations; visceral manifestations, almost countless in variety, but usually associated with cardiovascular changes, often with respiratory symptoms such as paroxysmal dyspnea and acute pulmonary edema, or gastrointestinal symptoms as vomiting, hiccough, and diarrhea; or urinary symptoms, observed in the clinical laboratory; dermal manifestations, such as pruritus, "dead fingers," pasty or so-called "cold buckwheat cake" appearance to the skin; and, finally, mixed manifestations, which may appear in almost countless variety. He speaks particularly of terminal pericarditis and of fever associated with uremia.

He describes the motor disturbances, particularly monoplegia, hemiplegia, and aphasia, as usually transitory and functional in character, although indistinguishable while present, from those produced by such organic changes as thrombosis or hemorrhage. Such cases at autopsy, showed no lesion, other than advanced kidney disease, which could have been held responsible for the paralysis. We have no clear knowledge as to the manner in which these transitory functional brain disturbances are brought about. It is possible that the poison acts directly on the brain cells, or it may act primarily on the blood vessels, causing spasm and cerebral ischemia.

Hamman takes exception to the inclusiveness of Riesman's enumeration of uremic symptoms. He defines the classical symptoms as lethargy, gradually progressing to coma, increased reflexes and muscular twitchings, stertorous breathing and often hyperpnea, anorexia and vomiting, a urinous odor to the breath, headache, increase in blood pressure and a predisposition to certain infections, chiefly stomatitis, enteritis and pericarditis. Convulsions, transient cerebral symptoms, psychic disturbances, paroxysmal dyspnea, pulmonary edema, Cheyne Stokes breathing and such other symptoms are often associated with uremia, but they occur still more frequently in the absence of renal insufficiency and are not necessarily due to uremic intoxication. This latter group, associated particularly with vascular changes, Hamman classifies as pseudoremic symptoms. They occur chiefly in association with hypertension and are often preceded by increases in blood pressure, the so-called blood pressure crises.

W. Langdon Brown classifies uremic symptoms as (1) cerebral, (2) respiratory, (3) gastrointestinal, (4) cutaneous, (5) muscular. He attempts to simplify this grouping. Thus the gastrointestinal symptoms, such as nausea, vomiting and diarrhea, although possibly sometimes central in origin, may result from attempts to find alternative channels of excretion. Eight grams of nitrogen may be excreted daily through the bowels. Canti, in a severe case of uremia, found the urea concentration in the blood to be 0.3 per cent, while in the vomitus it was 0.6 per cent. The skin manifestations may result from a similar process and myoclonus may be due to local intoxication as well as to a central disturbance. Thus Brown attempts to restrict the uremic syndrome to cerebral and respiratory manifestations.

He differentiates between uremia from chronic partial impairment of excretion, and urinemia, following complete suppression as from bichlorid of mercury or phenol poisoning. Urinemia is manifested by bodily weakness and languor, with few other symptoms. The symptoms of uremia do not occur in these cases, according to Brown, because fatal termination is so rapid that not sufficient uremic poison accumulates in the system. He suggests that incomplete excretion may lead to altered metabolism with the formation of abnormal toxic products, and defines uremia as a disease of metabolism, resulting from damaged kidneys. The cause of diabetic coma is not the retained or increased sugar of the blood, nor is the uremotoxin a retained urinary element. In both diseases the terminal symptoms are due to profound metabolic disturbances with the formation of a new poison. Golla suggests that in uremia the altered metabolism leads to the formation of trimethylamine, which he found increased tenfold in the blood in uremia, and that the hypothetical poison is closely related to trimethylamine. This view is not universally accepted.

Brown differentiates three forms of the acute nephritides. The first or toxemic kidney is distinguishable from a true nephritis by the fact that the urine rarely contains polymorphonuclear leucocytes, that the ratio between albumin and globulin is two to one as contrasted to six to one in true nephritis, that the output of diastase in the urine is low in nephritis but high in toxemic kidney, and that the blood urea is raised in the latter but not in the former. The blood sugar tends to show similar variations. In the toxemic kidney we have to deal not so much with a retention of excretory products as with an increased permeability of the kidney to anything which the blood delivers to it.

In the second form, or true acute nephritis, leucocytes are usually present in the urine and other differences are as stated above. The disease is often associated with infection elsewhere. Renal permeability tests show retention of chlorides and of urea and a diminished diastase output. Rarely did uremic manifestations occur in this type of nephritis.

The third form of acute nephritis or focal embolic nephritis is due not merely to the local action of a generalized toxin but to the actual deposition of infective microorganisms in the kidney. Here again, uremic symptoms rarely occur.

Among the classes of chronic nephritis, Brown calls attention particularly to what he terms "leaky kidney," a kidney which has suffered from an earlier but nonprogressive lesion. It allows proteins to escape into the urine but does not retain those substances which should be excreted and there are rarely associated cardiovascular changes. The ratio of albumin to globulin is more apt to be that found in toxemic kidney (2-1) than that of true nephritis (6-1). Renal permeability tests and the diastase output are normal. Uremic symptoms do not occur in leaky kidney, and the disease is compatible with fairly normal health despite the large amount of protein in the urine.

The point which Brown emphasizes in this discussion is that it is not the material which escapes but that which is not excreted which leads to uremia.

In an individual bordering on uremia or one in which the uremic poison is at work, sudden fluid depletion may so increase the concentration of the poison as to precipitate uremia. Thus in one case a diarrhea rapidly terminated in uremia. Water intake insufficient to maintain an adequate blood volume, when the kidney is incapable of excreting a concentrated urine may also predispose to uremia.

Brown states elsewhere that the symptoms of uremia may occur under conditions in which the kidney is not retaining toxins, such as in the toxemic kidney. This is somewhat at variance to his preceding statement. He suggests that it may be that the liver, through its inability to properly destroy the uremic poison, plays a greater part even than the kidney, in the production of uremia.

With regard to the suggestion that the drowsiness of uremia may be attributed to the acidosis that follows failure to excrete acid sodium phosphate, analogous to the acidosis of diabetes, criticism is made that even in diabetes it is not the acidosis *per se* that causes coma. The symptoms in the latter disease have been shown by Hurtle and Trevan to be due to the enolic group CO-COH, present in diacetic acid and they have shown that neutral sodium diacetate will produce the symptoms of diabetic coma equally well.

Geoffrey Evans' conception of nephritis may be summarized as follows: The kidney may be affected by pathogenic agents of varying kind and intensity, all of which, however, are capable of inducing active inflammatory reaction in the tissues they attack, whether epithelial or vascular. When acute, the epithelial reaction dominates the picture; when chronic, both epithelial and vascular changes co-exist; in the very chronic, only the vessels suffer, the pathogenic agent being too feeble to evoke reaction in the epithelial tissues, or these tissues being too feeble to react, they simply atrophy or degenerate. From this point of view neither vascular nor renal disease is to be regarded as the cause of the other, but both are due to the action of the same toxic agent.

From this partial review of work in this country and abroad, we observe that the conception of nephritis and uremia is changing from that of a local anatomical and functional disease, to that of a general metabolic disturbance in which the renal and urinary findings are secondary to a more profound intoxication. There appears still to be considerable uncertainty in the minds of those interested in the subject, as to whether the metabolic change producing uremia is incited by a primary kidney disease, or whether the renal pathology is entirely secondary to an antecedent generalized poisoning.

#### REFERENCES

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—W. T. V.



*Proposed Changes in the Medical Curriculum*

AT THE recent annual conferences of the American Medical College Association and the Council on Education and Hospitals of the American Medical Association certain proposed changes in the medical curriculum were discussed. No definite action, so far as I can ascertain, was taken. However, numerous advocates of the proposed changes were in evidence. The first proposition is founded upon the belief that medical students, especially during the first two years of the course, are worked too hard. From many years' experience as a teacher in a medical school I am inclined to the belief that there is truth in this proposition. The question is whether it would be beneficial to medical students to lessen their labors during these years. Suppose that the number of required working hours is reduced, to what extent would the students be benefited by such reduction? Students now entering our best medical schools may be divided into two classes. First, those who are unable, for various reasons, to comply with the strenuous demands made upon them. My experience is that this class makes up from thirty to forty per cent of those matriculating. It is highly advantageous to the good students that these "lame ducks" should be thrown out early in the course. Would this happen if the required hours were greatly reduced? I believe that first-year medical students should be submitted to a most strenuous test and that all halt, lame and generally incompetent should be discarded, preferably by the end of the first semester, certainly by the end of the first year. The second class of our present-day medical students consists largely of those who want to work all the time. It is difficult to drive them out of the laboratory. They desire to work more hours every day than the rules permit. They are excluded with difficulty on Saturday afternoon and some of them find excuses for admission on Sunday. The most serious objection that I see to reducing the required hours in the first year, or the first two years, lies in the fact that the unfit will be continued in subsequent years and many of them will ultimately be allowed to graduate.

Another proposition put forward at the meetings referred to, advises that clinical teaching be advanced to the second and even to the first year. The advocates of this proposition hold that a deeper practical interest in medicine on the part of the student will be secured in this way. At least one proponent of this plan speaks of it as an innovation—as something new in the manner of teaching medicine. This certainly is not true. Recent explorations establish the fact that Esculapius was not a wholly mythical personage, that he lived about thirteen centuries before Christ, that he and his followers established hospitals such as that unearthed on the Island of Cos, and that these were used as medical schools. In the temples of Esculapius young men prepared themselves for the medical profession by studying under teachers the symptoms and treatment of disease in the hospital patients. The clinical teaching of medicine is as old as the profession itself. In the temple of Esculapius at Cos the young man seeking admission to the medical profession had no instruction in chemistry, biology or physics, except that he did get some

information about mineral and vegetable drugs. Under this system, medicine was a trade, not a profession. Students were apprentices, and this system of teaching medicine continued until the latter part of the nineteenth century. During all this time clinical medicine predominated in medical schools. There was no requirement of courses of science set for the matriculating medical student. In the late seventies I spent two years in a medical school. I attended clinics from the first day of entering the school. In the school which I attended there was at that time unusual attention given to chemistry, physics, and biology; indeed, this special school was held up by its admirers as particularly efficient in the scientific branches, and by the mass of medical teachers in this country it was condemned on account of the paucity of its clinical material. The majority of the students entering this school at that time had no knowledge of the fundamental facts of chemistry, physics, and biology. Compared with other schools of that time its students had unusual laboratory opportunities. Still, clinical medicine dominated the curriculum and the first-year student spent a large part of his time in having patients presented to him and their symptoms and treatment discussed before him. Certainly the proposition to advance clinical teaching to the freshman year cannot be considered an innovation. Clinical teaching without previous scientific knowledge is as old as the profession of medicine; indeed, as I have already stated, there was no profession, there was a trade. The medical student acquired what skill he could by observation on patients. Some of these students developed into skilled practitioners, just as some artisans become highly skilled workers in wood and in metal. In the first week of my course in a medical school an able clinician presented a patient, with the following statement: "Gentlemen, this is a case of ascites. Ascites is an accumulation of fluid in the abdominal cavity and may be due to disease of the heart, liver or kidneys." Looking over the shoulder of the student who sat in front and below me I read his notes, which ran as follows: "Ascites is an accumulation of hearts, livers and kidneys in the abdominal cavity."

At the recent conference of the Council on Medical Education an advocate of the plan of advancing clinical teaching to the first year said that it would be greatly to the interest of the student to bring before the class cases of tuberculosis, kidney disease, and diabetes,—that such a presentation would give to the freshman student a deeper, more direct, and more intense interest in medicine than he now acquires in the laboratories of bacteriology, physiology, anatomy, histology, pharmacology, etc. Imagine the great and clear insight into medicine that would be acquired by presenting to the freshman medical student a case of diabetes. The clinician would say, this is a case of diabetes. Diabetes is usually due to some derangement of the pancreas. The special part of the pancreas affected in the development of diabetes is to be found in the islands of Langerhans, etc. The student who does not know whether the pancreas is behind the ears or in the ankles, who does not know whether it is composed of bone or muscle or mixed, who has no conception of the islands of Langerhans, would be greatly benefited, would he not, by this talk. In my opinion, this proposition to advance clinical teaching to the

first year is absurd in the highest degree unless certain other changes are made. It is possible to demand knowledge of the fundamental medical sciences for admission to the medical schools. This is possible, but we have heard no proposition to do this. Besides, it is not likely that this will be done. It is, of course, possible to advance the requirements for admission to medical schools to three or even four years in college. This is a proposition which has two sides to it and which may be worthy of discussion.

According to my understanding, modern medicine consists in the application of certain discoveries in physics, chemistry, and biology to the prevention or treatment of disease. Modern clinical medicine cannot be taught to those who are ignorant of these fundamental sciences. An hour or two a week of clinics given to first-year medical students would doubtlessly greatly interest them. I have my serious doubts as to the benefit that either student or patient would receive from these clinics. Admitting that medical students of the first and second years are overworked in the laboratories, need some change and recreation, and would enjoy a break in the day's work, I suggest that an hour a day be devoted to vaudeville. No doubt the students would be pleased, patients would not be harassed, the time of the clinician would not be lost, and the student would return to his laboratory work refreshed. Besides, this would not involve the return to the old medical curriculum hoary with three thousand years of age. The assumption on the part of those seeking to establish clinical teaching in the first year of medicine seems to be that the present medical curriculum, which has been tried for not more than thirty years, has proved a failure. In my opinion, the scientific teaching of medicine in which the student first becomes acquainted with the facts of physics, chemistry, and biology has advanced the profession more than did the three thousand years during which the whole of the medical curriculum consisted of clinical teaching. While we still have many incompetent physicians, I believe there was never a time in the history of the world when our profession could number in its membership such a large proportion of competent and efficient practitioners. However, I should not take this matter seriously because I do not apprehend that the so-called innovation in the medical curriculum, provided it is adopted, will accomplish anything more than to waste one or two hours of the time per week of the first and second-year medical student.

—V. C. V.

## BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

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### *Coleman's Materia Medica for Dentists\**

THIS is a small book and therefore cannot be comprehensive. As a matter of fact, it is really a compend, tabulating the characteristics and usages of each drug. Considered as such it is very good, but it would seem that even for the use of dental students the action of certain drugs at least, should be discussed in much greater detail.

At the end there are chapters on vaccine therapy, organo-therapy and electro-therapy. In the first of these, there is some confusion of identity between opsonins and antibodies. In the discussion of the action of the thyroid principle no mention is made of its effect on metabolism.

The volume, being in its fifth edition, has evidently proven its value.

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### *Practice of Medicine†*

THIS volume is a compilation of the work of twenty-six British authorities. Edited by Frederick W. Price. It is written as a textbook and should serve its purpose well. It is not intended as a reference volume and because of necessary brevity, it could scarcely be used as such. The editor points out that in his opinion, it is beyond the scope of any one authority, however varied his range of knowledge, to do full justice to the ever widening field of medicine, and it is for this reason that various subjects are discussed by authorities of unusual experience in each field. The book approaches nearer in composition to Osler's Principles and Practice of Medicine than to any other book. It is perhaps intended to take the place of the latter and can best be compared with it. Although of about the same actual size, being printed on thin paper and containing 1753 pages, it is really much larger. The editor has included sections on diseases of the skin, psychological medicine, and tropical diseases. The chief point of difference from Osler is that there are multiple collaborators. This fact has its obvious advantages, but

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\*Materia Medica for Dentists. Notes on Materia Medica Pharmacology and Therapeutics for Dental Students and Practitioners. By Frank Coleman, M.C., L.R.C.P., Assistant Dental Surgeon St. Bartholomew's Hospital; Dental Surgeon Royal Dental Hospital; Lecturer in Materia Medica to the London School of Dental Surgery. (University of London.) Cloth, Price \$3.25. Pp. 308. Fifth edition. Oxford Medical Publications. London. Henry Frowde and Hodder & Stroughton, The Lancet Bldg., 1 Bedford Street, Strand, W. C. 2. 1922.

†A Textbook of the Practice of Medicine, including sections on Diseases of the Skin & Psychological Medicine. By various authors, edited by Frederick W. Price, M.D., F.R.S. (Edin). Senior Physician to the Royal Northern Hospital, and Physician to The National Hospital for Diseases of the Heart, London; formerly Physician and Honorary Pathologist to the Mount Vernon Hospital for Consumption and Diseases of the Chest, and Examiner in Medicine at the University of St. Andrews. Cloth. Oxford Medical Publishers. Pp. 1753. London. Henry Frowde and Hodder & Stoughton, The Lancet Bldg. 1 Bedford Street, Strand, W. C. 2. 1922.



the volume loses the charm of personal touch so evident throughout Osler's *Principles and Practice*. It will make an excellent textbook.

## BOOK NOTICES

**COLLOIDS IN BIOLOGY AND MEDICINE**, by Prof. H. Beechhold, member of the Royal Institute for Experimental Therapeutics in Frankfurt A. M. Authorized Translation from the second German edition with notes and emendations by Jesse G. M. Bullowa A.B., M.D., Assistant Clinical Professor of Medicine, Fordham University; Adjunct Professor of Clinical Medicine, N. Y. Polyclinic School and Hospital; Visiting Physician Riverside Sanatorium; Associate Visiting Physician Willard Parker Hospital, N. Y. City. Cloth. Pp. 464, 54 illustrations. New York. D. Van Nostrand Co. N. Y. 1919.

**PHARMACEUTICAL AND FOOD ANALYSIS. A Manual of Standard Methods for the Analysis of oils, fats, waxes, and substances in which they exist; together with allied products**, by Azor Thurston, late chemist to the Ohio State Pure Food and Drug Commission. Cloth. Pp. 416. D. Van Nostrand Co. N. Y. 1922.

**THE PHYSIOLOGY OF TWINNING**. By Horatio Hackett Newman, Professor of Zoology University of Chicago. Cloth. Pp. 230. Chicago. University of Chicago Press. 1923.

**A TEXTBOOK OF THE PRACTICE OF MEDICINE**. By various authors. Edited by Frederick W. Price, M.D. F.R.S. (Edin). Senior physician to the Royal Northern Hospital and physician to the National Hospital for Diseases of the Heart, London. Formerly physician and honorable pathologist to the Mt. Vernon Hospital for Consumption and Diseases of the Chest, and examiner in medicine at the University of St. Andrews. Cloth. Pp. 1763. Henry Frowde & Hodder & Stoughton, Oxford Medical Publications, London, 1922.

**PRACTICAL PHYSICS**. By J. A. Crowther, ScD., F. Inst. P. Sometime fellow of St. John's College, Cambridge. Demonstrator in physics in the Cavendish Laboratory; University lecturer in Physics as applied to Medical Radiology, Cambridge. Cloth. Pp. 260. Henry Frowde & Hodder & Stoughton. Oxford Medical Publications. London, 1922.

**NOTES ON MATERIA MEDICA, PHARMACOLOGY AND THERAPEUTICS FOR DENTAL STUDENTS AND PRACTITIONERS**. By Frank Coleman, M.C., L.R.C.P., M.R.C.S., L.D.S., Asst. Dental Surgeon, St. Bartholomew's Hospital; Dental Surgeon Royal Dental Hospital. Lecturer in Materia Medica to the London School of Dental Surgery, (University of London). Fifth edition. Cloth. Pp. 308. Henry Frowde & Hodder & Stoughton, Oxford Medical Publications. London, 1922.

**LABORATORY TECHNIQUE, THE METHODS EMPLOYED AT ST. LUKE'S HOSPITAL, NEW YORK**. By F. C. Wood, Karl M. Vogel and L. W. Famulener. Second edition. Paper. Pp. 281. James T. Dougherty, New York. 1922.

**MODERN MICROSCOPY**. A handbook for beginners and students. By M. Cross and Martin J. Cole. Lecturer in Histology at Cooke's School of Anatomy. Fifth edition. Revised and rearranged by Herbert F. Angus. Cloth. Chicago Medical Book Co. Chicago, Ill. Pp. 315 with numerous illustrations and diagrams. 1922.

## Erratum

In Dr. Mirkin's article in the February issue, page 338, the thirteenth line from the bottom of the page should read:

$$X = \frac{541 \times 579 \times A - 579 \times 563 \times 2.7 \times V}{541(579 - 563)}$$

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## ORIGINAL ARTICLES

### NEW POINTS IN PHYSIOLOGY OF DIGITALIS THERAPY\*

BY ORVILLE HARRY BROWN, M.D. Ph.D., PHOENIX, ARIZ.

DIGITALIS has become more efficacious in my hands as the years of my practice have grown. The increased efficiency has been due to two factors, to wit: The use of larger doses than formerly and a better understanding of the physiologic indications for digitalis. Some of the data I present I believe not to be in the literature and original with me.

The advantage of larger—massive—doses of digitalis has been reported by various physicians. The late Dr. George Crandall of St. Louis was the first to teach me that the administration of digitalis should be in amounts necessary to get results rather than in conformity with dosage tables.

In the winter of 1910 I was caring for a physician's wife who had pneumonia. Her heart action was unsatisfactory to such an extent that it seemed that except for the free use of various emergency stimulants there were numerous occasions when she was near death. I had already given her digitalin, the use of which at that time was my custom, in  $\frac{1}{20}$  grain doses at four-hour intervals for 48 hours or longer; but there was no definite response on the part of the heart to this amount of digitalin. The average dosage of digitalin was given as  $\frac{1}{100}$  grain. I felt that giving five times the average dose of digitalin was near the limit of safety and feared to give more even though the indications for it were plain. Doctor Crandall was called in consultation and after examining the patient he advised digitalis. I told him that she had had repeated doses of digitalin each five times the ordinary dose; and he replied, "All right, give her twenty times the ordinary dose." We did. A fifth of a grain of digitalin worked results that seemed almost miraculous, and the smaller doses

\*Received for publication, January 16, 1923.

of digitalin thereafter maintained the heart in a fairly normal state during the next five to seven days to the crisis of the pneumonia.

I gradually came to using tincture of digitalis, not specifying the preparation, in thirty drop doses at short or long intervals as indicated. In severe cases I may give an initial dose of a dram or even more; but I prefer to give not over a dram and repeat in a relatively short time if necessary. Not infrequently I give thirty drops three times a day for a number of days. Except in the unusual case thirty drops twice a day for a week or possibly for two weeks and then thirty drops once a day for a week to a few weeks will usually give the desired effect. After the heart has reached the state desired smaller doses will usually keep the heart in good condition; or the thirty drops may be administered three or less times per week.

The one development which calls urgently for digitalis is cardiac dilatation. The best indication for cardiac dilatation, in the absence of fluoroscopic examination, is the location of the apex beat. When the apex beat is definitely more than eight centimeters to the left of the median line of the sternum, other causes for its dislocation being eliminated, there is usually some grade of dilatation of the heart. In subjects with small hearts there is certainly some dilatation at eight centimeters. The further out the apex beat is from normal the more urgent the demand for digitalis. It is just as important to know, too, if there is a dilatation of the right heart; but diagnosing this is more difficult.

In examining for dilatation one should use inspection, palpation, percussion, and auscultation, confirming the results of one examination by each of the others. In my own experiences auscultating for the point where the sounds of the heart first have a pronounced increase in intensity has proved to be the most reliable index in locating the borders of the heart—except in those cases where the apex beat is readily seen or definitely palpated. Cardiac murmurs are no criterion as to the needs of a heart for digitalis.

The musculature of a heart that is dilated receives less blood through its coronary arteries than does the muscle of the same heart when it is of normal size. Were a heart spherical a certain increase in its diameter would cause 3.1416 times as great an increase in its circumference. The ratio between the diameter and the circumference of a heart probably approximates that of a sphere. The coronary arteries, as a heart dilates, stretch in length approximately the same amount as the circumference of the heart is increased. An elastic tube stretched lengthwise decreases in diameter. I know of no law that would apply to the ratio of decrease in diameter with definite amounts of stretching in the length of elastic tubes, even of rubber, and certainly not of tubes of human tissue.

In order to establish some figures (by a method admittedly crude) as to what might be the decrease in coronary arteries stretched over a dilated heart I took the following:

A heart normally 100 mm. in diameter is dilated to 150 mm., and this is not an extreme case: the circumference would be increased from 314.16 mm. to perhaps 471.24 mm.; a heart normally 110 mm. in diameter is dilated to 140 mm.; the circumference would be increased from 345.57 mm. to 439.82

mm. Nine pieces of rubber tubing, some quite fresh and live and others old and relatively poor in elasticity, of varying diameters were stretched so that 314 mm. of the unstretched were brought to 471 and again so that 345 mm. of the unstretched tubes were brought to 440 mm. The length of the tube unstretched and stretched, the diameters of the tubes when unstretched and when stretched with percentages of decrease in diameter due to the stretching, with the averages, are shown in Tables I and II.

TABLE I

LENGTH UNSTRETCHED	LENGTH STRETCHED	DIAMETER UNSTRETCHED	DIAMETER STRETCHED	DECREASE IN MM.	PER CENT
314.16	471.24	10.5	8	2.5	23.7
"	"	9.5	7.5	2	21
"	"	7	5.5	1.5	21.4
"	"	6	4.75	1.25	20.8
"	"	5	4	1	20
"	"	4	3	1	25
"	"	4	3	1	25
"	"	4	3	1	25
"	"	3.5	2.5	1	28.5
"	"	Average			23.6

TABLE II

LENGTH UNSTRETCHED	LENGTH STRETCHED	DIAMETER UNSTRETCHED	DIAMETER STRETCHED	DECREASE IN MM.	PER CENT
345.57	439.82	10.5	9	1.5	14.3
"	"	9.5	8	1.5	15.8
"	"	7	6	1	14.3
"	"	6	5	1	16.7
"	"	5	4.25	.75	15
"	"	4	3.25	.75	19
"	"	4	3.50	.50	12.5
"	"	4	3.25	.75	19
"	"	3.50	3.	.50	14.3
Average					15.6

A decrease of 23.6 per cent in the diameter of a tube (or blood vessel) causes a 41.6 per cent decrease in cross sectional area; a decrease of 15.6 per cent in the diameter causes a 28.7 per cent decrease in cross sectional area. Then too as the diameter of a tube or blood vessel decreases, the friction between the liquid, passing through the tube or blood vessel, and the wall increases.

To show that stretching of capillaries causes a lessened blood supply take hold of a fold of the skin and put it on a stretch and observe the resultant pallor. It takes but relatively little stretching to make a noticeable change in color.

Since the coronary arteries are variable in diameter in various parts of any one heart and in different hearts it is not possible to apply a formula to them with any degree of accuracy; but by the figures of the above paragraphs and tables it is evident that a heart, dilated to only a moderate extent, may so stretch its coronary arteries that they may carry perhaps only 50 per cent



or less of their normal quotient of blood. The musculature of a dilated heart, even of a mild grade, does its work then while in an undernourished state. In such a heart digitalis, if given in sufficient dosage and the heart's condition is not so severe that the heart is past response, should cause a return of the heart to normal size, and the coronary arteries to their full blood carrying capacity. Another point not to be ignored in this connection is that with a dilated heart there is a tendency for a fall in the blood pressure on which the coronary circulation depends and digitalis counteracts this.

The usual teaching regarding the use of digitalis in pneumonia is that it should be withheld until there is a definite indication for it as shown by a fall in blood pressure and an increase in pulse rate. In my own opinion this is wrong. The argument advanced in favor of withholding the digitalis until plainly indicated, is that giving it before there is evidence of heart failure is like whipping a tired horse before nearing the end of the journey. I say, however, that giving the digitalis is not like whipping a tired horse but like feeding him; and one should not wait until near the end of the journey to feed the horse. In pneumonia the right side of the heart is put to an unusual test doing more work than usual and, when there is a dilatation, the entire heart has less nourishment than it has for its ordinary work. Therefore dilatation should be anticipated and digitalis should be begun coincident with the first intimation that there is a pneumonia. I am not sure but what this same advice should apply to a greater or less degree to even the chronic lung infections. I have found digitalis to be of extreme benefit in many cases of asthma, of tuberculosis, and other infections.

Another class of patients to whom I make it a rule to administer digitalis is the preoperative. A few doses of digitalis before the patient takes the anesthetic may save much grief during and following the operation. One patient who has been operated twice in the past few years had no digitalis before her first operation which kept her on the table for about an hour; she was badly cyanosed during the latter part of the operation, her apex beat was well out from the normal position and her recuperation was slow; before the second operation she had digitalis, was on the table for two hours or longer, came through the operation in good color and condition and had a rapid convalescence. I know full well that other factors than the use of the digitalis in the one instance and the nonuse in the other may have been active in causing the difference in the results in the two operations but I believe that the digitalis deserves much of the credit for the excellent condition in the second operation which was not present in the first. I have had patients suddenly collapse under anesthetics, whom I now believe would not have if digitalis had been administered before the operation. In some cases it seems wise to give the patient digitalis immediately following the operation, while he is still asleep, or if awake, before anything can be taken by the stomach; in such cases I add a dram or more of the tincture to the proctoelsis solution.

Many persons complain of slight dyspnea on exertion; in most of these no organic heart trouble can be found and at the time of examination no cardiac dilatation exists. In such cases it is probable that there was undue cardiac

dilatation at the time of the exertion which would have been easily found if examination had been made at the time, but which has disappeared at the time of examination. The first case which attracted my attention to this phenomenon was a young baker who came to me complaining of pain in the cardiac area and dyspnea on Tuesday, Thursday and Saturday. I learned that on these days he was doing heavy work and finally concluded that he was producing a temporary heart strain by the heavy work. Digitalis gave him relief. To individuals subject to heart strain it is wise to have them take digitalis when such exertion is anticipated; if it is not anticipated and the exertion is performed then have them take the digitalis as soon after the exertion as possible. There are times when any individual might well take this advice with profit. The heart muscle does any work demanded of it provided it has been carefully and systematically trained up to that amount of work. The athlete, however, is the only class of individuals who systematically and carefully trains his heart for periods of sudden strain. Even he, I presume, might find a few doses of digitalis, preliminary to hard contests, advantageous.

#### SUMMARY

The heart is an organ which may be subjected to exertion for which it has not been properly trained. The serious effect of overexertion on the heart is dilatation. As a heart dilates the coronary arteries are stretched in length and narrowed in diameter with the consequence of a reduced blood carrying power which may easily reach 50 per cent or more. At such times, then, the heart is doing more work than ordinarily and with less than usual nourishment. A heart that is dilated or in danger of being dilated needs digitalis, and in amounts to overcome or prevent the dilatation. The amounts needed may be much more than what has commonly been considered the proper dosage.

## HYPERGLYCEMIA—BASED UPON A STUDY OF 2000 BLOOD CHEMICAL ANALYSES\*

BY HENRY M. FEINBLATT, M.D., BROOKLYN, N. Y.

THE present paper represents an attempt to correlate some of the clinical evidences with the blood chemical picture in patients showing hyperglycemia. At the outset it was hoped that two highly important questions might be answered. What conditions other than diabetes mellitus may an abnormally high blood sugar suggest? What is the relation between hyperglycemia and glycosuria; i. e., how high must the blood concentration be before sugar appears in the urine, and is this threshold constant? A study of the tabulation soon convinced me that the data presented therein could give no definite answer to these questions. Thirty-four of the hyperglycemic patients studied presented clinical evidences of diabetes mellitus; forty-seven were considered nondiabetic in spite of their hyperglycemia. In this latter series the diagnoses included a heterogeneous group, and, with a few exceptions, could not account for the blood sugar values attained.

The difficulty in reconciling the blood sugar readings with the results obtained from the examination of the urine arises largely from the fact that the two specimens are seldom taken simultaneously. Blood sugar values oscillate widely and rapidly. Today a reading of 1000 mg. per 100 c.c. may be obtained; a few days hence, the figure may be down to 250 mg. This fact would appear to explain in part some of the discrepancies noted in this respect.

The two leading questions of practical importance cannot be answered on the basis of the cases here studied, but incidentally the analysis brings to light some points of considerable interest.

Most of our knowledge of blood chemistry has been acquired during very recent years. Not so with the subject of blood sugar. Quantitative determinations were made by Claude Bernard quite some years ago. In his *Leçons sur le Diabète*<sup>1</sup> he described the method in detail. While the technic was laborious and 25 c.c. of blood were required, his results were apparently quite accurate. He reported the normal figure for man as 90 mg. per 100 c.c., which figure still stands.

As regards the more recent methods, the usefulness of Benedict's test appears to be vitiated by the fact that the readings seem to be falsely elevated when there is considerable retention of creatin and creatinin. Experimenting by adding known amounts of creatin and creatinin, I was able to satisfy myself that this objection does not apply to Folin's test. The great majority of sugar determinations here reported were made by Folin's method.

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## MATERIAL STUDIED

Out of 2000 routine blood sugar determinations taken in the wards of the Long Island College Hospital, there were separated all of those cases, regardless of diagnosis, which exhibited a reading in excess of 150 mg. per 100 c.c. Complete blood chemical analyses were made upon medical cases as a matter of routine. This scheme of mechanical winnowing must obviously yield results which are entirely free from the prejudices of the writer. Because of the large number of cases the findings should be fairly representative.

The data upon all of the hyperglycemic patients were tabulated, but because of its great length this table has been omitted. Altogether 81 patients yielded blood sugar readings in excess of 150 mg. per 100 c.c. of blood.

TABLE I.

SUMMARY OF CLINICAL DIAGNOSES IN THE 81 HYPERGLYCEMIC PATIENTS	
Diabetes mellitus	34
Hyperthyroidism	3
Cerebral hemorrhage	3
Chronic glomerular nephritis	3
Menopause	3
Chronic bronchitis	3
Rheumatic fever	2
Neurosyphilis	2
Syphilitic aortitis	2
Miscellaneous conditions (occurring only once)	26

The outstanding features of this chart are (1) the finding of clinical diabetes mellitus in 42 per cent of the patients who showed hyperglycemia, and (2) the heterogeneous nature of the clinical diagnoses in the other group of 47 apparently nondiabetic individuals, representing 58 per cent. The presence of an abnormally high blood sugar concentration can be explained by the diagnosis in only a small percentage of the nondiabetics.

Various endocrinopathies, particularly hyperthyroidism, hyperpituitarism and hyperadrenalism, have repeatedly been demonstrated to exhibit hyperglycemia and glycosuria. Consequently, this finding in three cases of hyperthyroidism is not remarkable.

Glycosuria has frequently been observed in organic diseases affecting the brain, particularly in the region of the ventricles or the hypophysis. Claude Bernard in 1849, in his famous piqûre experiment, first demonstrated the influence which the nervous system may exert in the production of glycosuria. By puncturing a spot on the floor of the fourth ventricle, he was enabled to induce an artificial diabetes in the dog. Although this experiment has not been confirmed in man, Osler's case, seen with Reiss at the Friedrichschain, Berlin, of a woman with anomalous cerebral symptoms and diabetes, in whom at necropsy a cysticercus was found in the fourth ventricle, furnishes an analogy. Under the caption of brain lesions would fit our three cases of cerebral hemorrhage, the two of neurosyphilis, the one of epidemic encephalitis, and the one in which postmortem examination disclosed a dural tumor in the neighborhood of the pituitary body.



In three individuals the high sugar reading appeared to be attributable to chronic glomerular nephritis. The figures in these cases were 210, 181, and 153 mg. per 100 c.c. respectively. The coexistence of moderate hyperglycemia with nitrogen retention is quite usual. The apparent sugar values as estimated by Benedict's method are raised by creatin and creatinin, giving false readings where there is considerable retention of these substances. In Folin's method the sugar readings are not influenced by the amount of creatin and creatinin present. A study of twenty-nine cases of uremia by the author<sup>2</sup> showed that abnormally high sugar values are frequently associated with nitrogen retention, but that this finding is far from constant; in fact, normal and subnormal sugar values are quite compatible with maximal grades of nitrogen retention. Even in cases in which the urea nitrogen rose to 375 mg. the sugar values were frequently within normal limits. In those cases in which the sugar content was above normal, this finding was apparently due to increased tolerance rather than to retention. The author<sup>3</sup> investigated 43 cases showing retention of creatinin. Here moderate hyperglycemia was frequent, but the figures bore no proportion to those for creatinin, and subnormal sugar values were often found together with high-grade creatininemia.

Folin<sup>4</sup> in 1920 discussed this phase in a Harvey lecture as follows:

"In this connection I would call attention to the highly peculiar fact that bloods from nephritics having very high urea retention give by our original method, as by all other sugar methods, abnormally high values for the blood sugar. There does not seem to be any tangible reason why such bloods should contain any more sugar than the blood of normal individuals. There is room for suspicion that in such bloods other materials than sugar play an important part, that similar products in smaller amounts are present in all blood, that all sugar values obtained are high and that the lowest sugar values obtained must still be regarded as maximum values. We know, of course, that creatine and creatinine tend to raise the apparent sugar value of blood by Benedict's method. These products, however, do not have any such effect in our method, yet by our original method we did obtain abnormally high sugar values in bloods containing 150 mg. or more of nonprotein nitrogen per 100 c.c. of blood."

One patient in the nondiabetic series was suffering from a manic-depressive psychosis, and in three the symptoms associated with the menopause were so serious as to require admission to the hospital. This leads up to what I now regard as the keynote of those obscure hyperglycemias, viz., the emotional factor.

Since the impressive researches of Cannon, the part which the emotions play in affecting sugar metabolism has been thoroughly appreciated. In one experiment, Cannon<sup>5</sup> bound twelve cats, in all of which the urine was sugar-free, to a comfortable holder, leaving the head loose. All of the animals developed a well-marked glycosuria, the average time being one hour and a half. In those cats which stood the test calmly, the sugar was late in appearing; whereas in the animals which were perturbed, early glycosuria developed. On the day following the experiment, the urine of all of the cats was sugar-free.

If hyperglycemia and glycosuria can be induced by frightening a cat or irritating a dog, or by subjecting a student to the mental strain of acting as a spectator in the football stadium, it is not strange that it should be found in hospital patients as a result of psychic factors. The clinical record makes no note of the mental perturbation caused by anxiety over the outcome of one's illness, or by a disturbing visitor, or the emotional upset resulting from exasperation with an orderly, yet these circumstances may exert a determining influence upon the blood sugar curve.

In the psychoses, particularly melancholia and depressive states, hyperglycemia is of high frequency. Kooy<sup>6</sup> in 1919 reported this condition in 19 cases of melancholia and 4 of neurasthenia and psychasthenia. He noted it less often in confusional insanities and marked emotional upsets, and occasionally in general paresis and dementia precox. Schwab<sup>7</sup> in 1922 studied blood sugar tolerance curves in neurologic conditions. He found a high frequency of abnormally elevated curves in the psychoses, particularly in melancholic and depressed states. The curves in themselves were not diagnostic of any particular psychosis, but merely appeared to be proportional to the degree of emotional disturbance. In the neuroses, increased blood-sugar values showed a tendency to be present in those individuals who were depressed and who were in the habit of worrying excessively.

Of 47 apparently nondiabetic individuals exhibiting hyperglycemia, the clinical record in 33 cases, or 70 per cent, could offer no adequate explanation. Some of these patients, of course, might actually have been bordering upon a diabetic state. This possibility cannot be ruled out without a careful and prolonged follow-up study. However, the findings obtained after a complete study in the hospital were not such as to justify the diagnosis of diabetes mellitus.

In one case, the diagnosis, prolapse of the uterus with cystocele, and the clinical record could in no way account for the high blood-sugar value, 400 mg. per 100 c.c., and the presence of a large amount of sugar in the urine. These findings were obtained on admission. The sugar promptly disappeared from the urine, and, eight days later, the blood sugar had receded to 142 mg.

An examination of the figures in the series of diabetic cases revealed marked and rapid fluctuations in sugar values. A blood-sugar reading applies only to the date on which the specimen is taken. It is remarkable to note how rapidly the blood sugar concentration falls under strict dietetic regimen. Today a reading of 1000 mg. per 100 c.c. may be obtained. Tomorrow, it may be 666 mg., and the following day, 333 mg. So far from handicapping us in our understanding of an individual case, this great liability of blood sugar level is of benefit to us. It enables us to gauge quite closely just how well the patient is handling his particular diet. To adjust the diet in the light of information obtained from frequent blood sugar determinations is to handle the case in the most intelligent manner possible.

The fact that hyperglycemia may result from emotional states necessitates caution in the interpretation of a blood sugar reading. Confusion may be obviated by keeping in mind the fact that, except in the psychoses where the

affective disturbance is more or less continuous, this type of hyperglycemia is transitory. Persistence is the criterion of its pathogenicity.

Failure to take fully into account the effects which psychic influences may have upon the blood sugar concentration may lead to false conclusions in the line of *post hoc, ergo propter hoc* logic. Frenkel-Tissot<sup>8</sup> in 1920 reported his studies upon young Austrian soldiers who had escaped from Italian prison camps and fled over the mountains back to Austria-Hungary. He concluded that altitude does not affect the blood sugar, but that the latter is elevated by sun baths and electric light. In view of the harrowing experiences through which these young men had just passed, it appears to me that emotional factors might have played no inconsiderable part in determining the results.

An interesting observation has been made as to the effect of a tropical climate upon the blood sugar. DeLangen and Schut<sup>9</sup> in 1918, from their extensive researches both in man and in animals, were enabled to demonstrate that the blood sugar value ranges from 30 to 75 per cent higher in Java than in Europe. Residence inland in the mountains reduced these figures, which, however, promptly returned to their former level when the subjects went back to the seashore.

#### RELATION BETWEEN HYPERGLYCEMIA AND GLYCOSURIA

The normal blood sugar value ranges from 90 to 110 mg. per 100 c.c. The concentration of sugar in the blood must rise considerably above the high normal figure before it will appear in the urine in such quantities as to be detectable by ordinary tests. It is very difficult to say exactly what this level is, because the blood sugar curve fluctuates so rapidly, and the urine cannot be obtained synchronously.

Claude Bernard<sup>10</sup> in 1877 gave the threshold figure as 0.3 to 0.35 per cent. Labbé<sup>11</sup> in 1920 gave it as 0.2 per cent. McCay<sup>12</sup> and his coworkers in 1919 came to the conclusion that the threshold stimulus of the kidney for sugar excretion varies widely in different individuals and in the same individual under different circumstances. They could find no parallelism or arithmetical proportion between the blood sugar and that found in the urine. They believed the normal threshold to lie somewhere in the neighborhood of 170 or 180 mg. per 100 c.c. In diabetics they found this threshold higher, and it rose progressively with the duration and increasing severity of the disease. Indeed, before death, blood sugar values often reached great heights without appearing in the urine. Cammidge<sup>13</sup> in 1920 studied 700 diabetics and could find no constant threshold value. Some patients with a permanently high blood sugar curve passed comparatively little in their urine; in others, normal or subnormal values in the blood were associated with frank glycosuria.

Our cases serve to emphasize the inconstancy of this relation between glycosuria and hyperglycemia. Thus, in one case, the blood sugar values ranged from 500 to 660 mg.; nevertheless two of the urinary reports were negative for sugar. Other cases are contained in the tabulation in which the blood sugar attained a level of 500 mg. and in which nevertheless there was no glycosuria. In only six of the forty-seven non-diabetic individuals was there a coexistent



glycosuria. Still the figures in a large proportion of these cases were far above the normal threshold. It is probable that in the majority of these patients the hyperglycemia was transitory. This might perhaps have been accompanied by a transient glycosuria, which was missed because the urine examined was not the urine secreted at that particular time. In others, the absence of sugar from the urine appears attributable to a high renal threshold.

## SUMMARY

Out of 2000 routine blood sugar determinations taken in the wards of the Long Island College Hospital, 81 yielded readings in excess of 150 mg. per 100 c.c. Thirty-four of these individuals, or 42 per cent, presented clinical evidences of diabetes mellitus. The remaining 47 patients were considered non-diabetic. A prolonged followup study might, of course, have proved some of these individuals to be diabetic; but a complete study in the hospital did not yield enough data to justify such a diagnosis. The nondiabetic series furnished a heterogeneous group, and in only 14 of these individuals, or 30 per cent, could the diagnosis account for the hyperglycemia. It is believed that a single report of an abnormally high blood sugar value cannot be construed as pathologic, inasmuch as transient hyperglycemia may result from emotional disturbances. A study of the relation between hyperglycemia and glycosuria served to emphasize the fact that the renal blood sugar threshold varies in different individuals and in the same individual under different circumstances. In diabetes a high threshold value may exist, and this tends to increase in proportion to the severity of the disease.

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## AN INVESTIGATION ON THE PICRAMIC ACID METHODS FOR BLOOD SUGAR\*

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SINCE Lewis and Benedict<sup>1</sup> first described their picramic acid method for the analysis of blood sugar, there have arisen from time to time various objections to it. Several modifications of this method have been described that have, in some cases, greatly improved it, while in others no advantage has been shown over the original method. The chief criticism has been that the methods (original and modified) do not give consistent results, and often give results that are too high. In a recent work one of the authors (H. C. S.<sup>2</sup>) demonstrated the ease with which certain substances interfere with the reaction and suggested the possibility of a similar interference in the test as applied to blood.

It is the purpose of this work to check these methods and if possible demonstrate any fault or virtue present. It is not especially the results in normal subjects but in pathologic ones that are of real significance, for the results in the relatively normal cases are not exaggerated enough to be of serious import. In the pathologic specimens, however, in which unknown reducing bodies or substances, such as phenol, may be present in large amounts, the possibility of error is increasingly great. It is in the pathologic subject that an accurate knowledge of blood chemistry is most desired.

### EXPERIMENTAL

After the standardization of the methods was completed it was planned to apply them (a) to a series of normal or nearly normal subjects, (b) to a series of pathologic subjects, including a series of animals after injections of various substances.

The more recent method of Benedict<sup>3</sup> and the Myers and Bailey<sup>4</sup> modification was selected as the most representative of the picramic acid methods. Along with them the two methods of Folin and Wu<sup>5</sup> were used, not so much for an absolute standard as for a comparative study. There is no reason why the Folin method may not give results that are too low as well as for the others to give results that are too high.

The technic of the new Benedict method is in brief as follows: Two c.c. of whole blood are laked with 2 c.c. of water and made up to 25 c.c. gradually and with gentle rotation with sodium picrate solution. This is filtered and to 8 c.c. of the filtrate is added 1 c.c. of 20 per cent sodium carbonate

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†As this work was nearing completion, this young investigator was taken with an illness that terminated in death. Her work was continued by Mr. Armada T. Weathers and Dr. K. Lucille McCluskey, to whom the surviving author acknowledges appreciation.

solution. After mixing, this is heated for ten minutes in a water-bath at 100° C. and read against a standard similarly prepared. The sodium picrate for this method is prepared by dissolving 36 gm. of purified picric acid in water, with the aid of exactly 16 gm. of sodium hydroxide, the solution is filtered, cooled and made up to 1000 c.c. It should have an acidity of 0.05 to 0.04 normal.

The Myers and Bailey method consists in laking 5 c.c. of blood with 10 c.c. of water and adding one-half a gram of picric acid; this is shaken thoroughly and diluted to a final volume of 25 c.c. with water. To 3 c.c. of the filtrate is added 1 c.c. of 20 per cent sodium carbonate solution the tube stoppered with cotton and heated for 15 minutes at 100° C. in a water-bath, cooled, diluted to 10 c.c. with water and read against a standard sugar solution similarly prepared.

The original Folin and Wu method is as follows: 10 c.c. of blood are laked with 70 c.c. of water, and to this 10 c.c. of 10 per cent sodium tungstate solution are added, followed by 10 c.c. of exactly  $\frac{2}{3}$  normal sulphuric acid. This is shaken and filtered; 2 c.c. of this filtrate are placed in a special constricted test tube, 2 c.c. of an alkaline copper solution are added and it is placed in a boiling water-bath for exactly 6 minutes, it is then cooled in a beaker of cold water, 1 c.c. of phospho-molybdic phospho-tungstic acid reagent is added, and then 5 c.c. of saturated sodium carbonate solution. This is diluted to 25 c.c. and allowed to stand 5 minutes and read against a standard similarly prepared.

Folin's new method is the same as the old, except that the tubes are taken from the water-bath and cooled in a beaker of water for 2 or 3 minutes without shaking. To this, 2 c.c. of the new molybdate reagent are added, allowed to stand 2 minutes, diluted to 25 c.c. and read against a standard similarly prepared.

In addition, a plan was used in which a definite quantity of sugar was added to each blood filtrate and the amount recovered was estimated. For example in the Benedict method, the sugar, or color-producing substance calculated as glucose, found in the 8 c.c. of blood filtrate which in turn represented 0.64 c.c. of blood, is, say, 0.6 mg.; the amount added is 0.5 mg. but the amount found by color comparison is, say, 1.2 mg. Therefore, 0.1 mg. is recovered in excess of the sugar originally present plus the added sugar. This is recorded in the table as a positive correction. The argument for this method of checking is that the same substances that will cause an augmentation or reduction of the sugar reading in the blood filtrate will have a like effect on the added sugar. This can only be true where the added sugar is present in small quantities, because the concentration of sugar has a varying effect on the reduction of alkaline copper or other such oxidizing mixtures.

A more rigid technic was used in the picramic acid methods, than was required by the methods as described. The reagents were added in the same sequence each time, with approximately the same time limit for each step in

the process. The water-bath was kept at exactly 100° C. by dissolving sufficient calcium chloride in the water, to compensate for any variation in pressure. After placing the tubes in the water-bath, the temperature was brought back to 100° C. as quickly as possible, by the use of accessory burners, because the tubes cooled the bath down from 3 to 6 degrees.

Accordingly, each determination was done in triplicate; to one tube of blood filtrate was added 0.25 mg. of sugar; to another 0.50 mg. of sugar, while the third was analyzed without the addition of sugar. The sugar was added in 0.1 c.c. of solution to the quantity of blood filtrates recommended in the respective methods. In the Folin methods the technic was slightly altered. One-half of the blood filtrate was used (i.e., 1 c.c.) and to this 0.05 mg. of sugar was added in 1 c.c. of solution, making a volume of 2 c.c. as called for in the method. The results obtained with this technic were checked with the original, i.e., 2 c.c. of blood filtrate were used for analysis to which was added 0.1 mg. of sugar in 0.1 c.c. of solution. Due to the fact that practically identical results were obtained by both methods, and the decreased amount of work required, the former technic was adopted.

The first group to be studied consisted of 33 patients who were selected at random from the ambulant patients of this institution. They were either incipient or moderately advanced cases of tuberculosis. So far as the known blood chemistry is concerned, they were normal. Complete chemical analyses were made for uric acid, urea nitrogen, nonprotein nitrogen, preformed creatinine, total creatinine, cholesterol and blood chlorides. In no case were any abnormalities observed, thus these results have been omitted. The results are averaged and tabulated in Table I.

TABLE I

AVERAGE OF THIRTY-THREE SAMPLES								
BENEDICT METHOD	MYERS METHOD	OLD FOLIN METHOD	NEW FOLIN METHOD	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.25 MG. ADDED TO BENEDICT TEST MULTIPLIED BY 100	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.25 MG. ADDED TO MYERS TEST MULTIPLIED BY 100	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.50 MG. ADDED TO BENEDICT TEST MULTIPLIED BY 100	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.50 MG. ADDED TO MYERS TEST MULTIPLIED BY 100	AMOUNT OF SUGAR RECOVERED MORE OR LESS THAN 0.05 MG. ADDED TO THE NEW FOLIN TEST MULTIPLIED BY 1000
105.7	99.8	100.0	94.5	+4.8*	+4.2	+10.5	+7.6	-1.5
				+4.2	+4.3	-7.8	-6.7	
				+1.8	+0.7	+4.5	+1.1	

\*This row represents an average of the plus readings; the next row an average of the minus readings; while the last is a general average of all.

The average results obtained on these 33 samples of blood show that these methods gave a figure of decreasing value in the order named:

Lewis and Benedict	105.7	mg.	sugar	per	100	c.c.	blood.
Folin and Wu method	100.0	"	"	"	"	"	"
Myers and Bailey method	99.8	"	"	"	"	"	"
Modified Folin and Wu method	94.5	"	"	"	"	"	"

The average results of the sugar determinations by these various methods on the blood filtrate before and after the addition of .25 mg. and .50 mg. of glucose are arranged in Table I under the proper heading.

The results obtained in the picramic acid methods when sugar was added were very erratic. A quantity sometimes greatly in excess and again less than the amount added, was indicated. The grand average, however, showed a plus recovery in the picramic acid method and a negative reading in the new Folin method. The recovery in the new Folin method though always low, was consistent and gave results more in accordance to theory.

In the next group a study was made on various pathologic bloods. These were obtained from private patients, patients from our institution and from the Cook County Hospital through the kind permission of Dr. Moody, Resident Pathologist, to whom we wish to acknowledge our appreciation. There are also added to this list results obtained on animals after injection of phenols.

Table II shows the results obtained.

The first three patients may be grouped together, as all had marked retention of urinary constituents with the attendant symptoms. The Folin tests are 30-90 per cent lower than the Benedict. Furthermore, by the picramic acid method the added sugar gave figures which indicated a recovery almost double in amount to that actually present. The next three patients (4, 5, and 6) were also nephritics having a retention of urinary constituents, but when sugar was added it was almost a complete recovery in patients 4 and 6 and only a 50 per cent recovery in patient 5. The next four patients were diabetics or of diabetic tendency. In Nos. 8 and 9 all the blood methods gave concordant results, but in No. 10 there was a gross discrepancy. This patient had a more or less mottled history including among other things, alcoholism, empyema, lung abscess, gastric ulcer, and far advanced pulmonary tuberculosis. Three weeks prior to death diabetes developed, with 7 per cent sugar in the urine. At autopsy all of these conditions were verified and in addition an aortitis and slight endocarditis. The blood chemistry analysis in addition to the results given in Table II was as follows. expressed in mg. per 100 c.c. of blood: total phenols, 4.3 mg.; total nitrogen 2995 mg.; fats, 467 mg.; amino-nitrogen 6.0 mg.; total creatinine 5.1 mg.; chlorides 430 mg.; inorganic phosphorus 2.5 mg.

The striking thing about this case is the divergence of the two types of blood sugar determinations. Duplicates were run on succeeding days to preclude the possibility of error. At the present time we have no explanation of the phenomenon. No. 11 was a patient having a profound jaundice from an "acute catarrhal" inflammation. No. 12 was a normal woman who developed an acute "toxemia" with constipation. Neither are unusual, but



TABLE II

NUMBER	INITIALS	CASE NO.	BENEDICT METHOD	MYERS METHOD	OLD FOLIN METHOD	NEW FOLIN METHOD	% INCREASE OR DECREASE OF TOTAL SGAR, WHEN 0.25 MG. OF SGAR IS ADDED TO BENEDICT TEST	% INCREASE OR DECREASE OF TOTAL SGAR, WHEN 0.25 MG. OF SGAR IS ADDED TO MYERS TEST	URIC ACID	NONPROTEIN N.	PHENOL	UREA N.	CREATININE	REMARKS
1.	M. E.	C. C. H. 812815	115.8	—	93.0	73.0	+49.0	—	2.6	—	—	39.7	2.0	Nephritis, hypertension, cardiac decompensation, ascites, impending coma
2.	C. C.	C. C. H. 814724	81.7	—	63.0	45.0	+48.0	—	2.7	41.2	—	—	1.5	Nephritis, lactae origin?
3.	A. M.	C. C. H. 812634	86.2	—	68.0	51.5	+68.0	—	3.4	—	—	20.1	1.7	Chronic myocarditis, nephritis with hypertension
4.	M. M.	C. C. H. 778160	153.0	152.0	—	(110.0 (111.0	+5.7	+6.8	8.3	—	—	97.5	7.5	"Syphilitic" nephritis
5.	Patient of Dr. F.	—	55.0	38.3	—	50.0	-19.0	-36.0	4.7	—	—	24.4	3.0	Nephritis, hypertension, "double mitral," coma
6.	Foster	C. C. H.	103.7	100.1	—	80.0	+1.7	+9.4	5.8	—	—	24.0	3.0	Nephritic
7.	P. O. T. 1 mo. earlier	—	92.0	96.0	(110.0 (222.0	81.0	+3.0	-5.4	(1.3 (4.1	—	—	19.5 14.8	1.8 1.5	"Prediabetic" (?)
8.	A. M.	C. C. H. 784195	375.0	400.0	—	411.0	+4.1	-0.3	2.2	—	—	11.5	—	Diabetic

TABLE 11—CONTINUED

NUMBER	INITIALS	CASE NO.	BENEDICT METHOD	MYERS METHOD	OLD FOLIN METHOD	NEW FOLIN METHOD	% INCREASE OR DECREASE OF TOTAL SUGAR, WHEN 0.25 MG. OF SUGAR IS ADDED TO BENEDICT TEST	% INCREASE OR DECREASE OF TOTAL SUGAR, WHEN 0.25 MG. OF SUGAR IS ADDED TO MYERS TEST	TRIC ACID	NONPROTEIN N.	PHENOL	CREA N.	CREATININE	REMARKS
9.	L. G.	C. C. H. 783417	922.0	183.0	—	208.0	+13.7	+10.5	2.6	28.2	—	12.6	1.4	Diabetic
10.	M. P.	7024	483.0 497.0	594.0 636.0	180.0 175.0	210.0 200.0	-1.8	—	—	—	—	13.2	—	Alcoholic, with lung abscess, empyema, "T.B.," peptic ulcer, diabetes
11.	A. C.	—	126.0	—	—	95.0	-1.8	—	—	—	—	—	—	Catarhal jaundice
12.	M. U.	—	168.0	—	—	—	+10.0	—	—	—	—	—	—	Normal case with "acute toxemia" and constipation
		Dog 11	97.3 165.4	126.0 202.0	61.5 100.0	53.5 93.0	+9.0 +40.0	+10.0 +35.0	3.6 3.6	—	—	—	—	Before Injection
		Dog 111	123.4 136.0	152.4 157.0	100.0	93.0	+13.4	+6.0	2.6	—	4.3	—	6.1	After Injection of 20c.c. of 2.5% phenol
														Before Injection
														After Injection of 10c.c. of 10% cold potassium phenyl sulphate, following which dog died of shock.
														Blood was taken from heart after death

it demonstrates that even in such patients the picramic acid tests may be unreliable.

In Dog 2, 20 c.c. of 2.5 per cent phenol was injected before and after which blood was drawn for analysis. The results are all that was expected. In Dog 3, 10 c.c. of cold potassium phenyl sulphate were injected and the dog died, apparently of shock. The second bleeding was done from the heart after death. These results are similar to those obtained in Dog 2. In Dog 3, total phenols, uric acid, and creatinine were determined.

#### DISCUSSION

It is evident that there are serious discrepancies between the picramic acid methods on the one hand and the copper reduction methods on the other. These discrepancies are not so marked in normal subjects and even in certain pathologic ones. In other pathologic subjects, however, the difference is too great to pass by without an investigation of the cause. After a rather exhaustive study, we have not been able to account for all this variation. At one time it was thought that phenols might be the principal interfering factor, but in patient No. 10, Table II, in which the phenols and the other known chemical constituents of the blood were normal, there was still almost a trebling of results by the picramic acid methods over the Folin methods. This same thing was true in the experiments on Dog 3, Table II. It may be that under certain abnormal conditions some products are formed from glucose, or some other chemical constituents of the blood, that will react in the picramic acid methods to give additional color. In that event, there will be an actual usefulness for these methods in determining the "picramic reducing substances other than glucose." Because of the nonuniform results in certain mixtures, (for example where phenol is present), these results cannot be standardized until more is known of the reaction.

In uncomplicated diabetes, there is no exaggeration of results by the picramic acid methods. In certain patients with grave nephritis and retention of urinary constituents, there is an actual diminution in the results by the picramic acid methods over the others. In one patient the added sugar was only half recovered. Possibly a combination of substances was present that suppressed the normal color development. In the majority of patients with nephritis and retention of urinary substances, however, there is a great augmentation of results by the picramic acid methods.

The merits of the check method devised need not be discussed here. The picramic acid methods gave results that were erratic and usually high while the Folin methods gave results that were consistent though slightly low. Possibly the true sugar value is a little above the results obtained by the new Folin method. There may not be complete oxidation of the glucose or part may be oxidized by the oxygen of the air. The average added sugar recovered was 3 per cent lower than that added. For reliability, accuracy, and simplicity it is far superior to any of the picramic acid methods. The excess results by the picramic acid methods may indicate the presence of substances that are not strong enough reducing agents to reduce copper oxidizing mix-

tures, yet are able to reduce alkaline picrate solutions by acting directly or indirectly on the alkaline picrate.

#### SUMMARY

(1) It has been demonstrated that the picramic acid methods for the estimation of blood sugar are unreliable in certain pathologic conditions.

(2) It has also been established that the presence of phenol or potassium phenyl sulphate in blood produces a false sugar result when these methods are employed.

(3) From results obtained upon pathologic subjects and by the addition of sugar to blood filtrates it would seem to indicate that the modified method of Folin and Wu for the estimation of blood sugar possesses many advantages over any of the other methods studied.

(4) It is suggested that the picramic acid methods may be used to determine the presence in the blood of "reducing substances other than glucose."

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## THE PRESERVATION OF NATURAL COLORS IN GROSS SPECIMENS\*

BY OSKAR KLOTZ, M.D., SAO PAULO, BRAZIL

IN recent years there has been a growing interest in medical museums, and they are being adapted more generally to the needs of student teaching. This is particularly true of the museums associated with the Department of Pathology, where it has been found that properly preserved specimens of pathologic lesions can be used to great advantage to instruct the students in the progressive changes which formerly were demonstrated only in the microscopic sections.

There are few schools where there is an autopsy clinic of sufficient proportion to rely upon the routine material to illustrate all the features of the diseases which are discussed in the various subdivisions of pathology. This is particularly true in the United States, and it is here that we find that the entire attitude to the pathologic museum has been revised. The museum is no longer a storehouse for rarities of disease or grotesque anomalies arising in clinical practice. The more modern museum deals with specimens illustrating the common diseases where the progressive processes of the tissue change can be studied in their macroscopic appearances. Thus at first sight it would appear that many museums have their shelves filled with duplicates, but on closer study we find that these similar specimens show variations in a common process, or show advancing stages of the disease. A well organized series of specimens showing the lesions in pneumonia, tuberculosis, appendicitis, cholecystitis, and in many other inflammatory processes, is invaluable for teaching purposes, not only within the Department of Pathology, but also for the clinical departments which are interested in the general exposition of their subject during the practical exercises. We know of no other method whereby human pathologic studies can be presented to the student in an equally convincing manner. In our own experience it has never seemed advisable to use this material during the didactic discussion of disease, but we have found it much more advantageous to link the study of the gross specimen at the time when the student was carrying on his histologic exercises. For the student to familiarize himself only with the macroscopic characters of inflammation without at the same time gaining a concrete idea of the general characters of the lesion from his inspection of gross specimens, seemed to us an incomplete and improper presentation of the subject. Illustrative specimens can be collected and prepared for almost every type of lesion with which we desire the student to familiarize himself.

Furthermore, we have found it extremely stimulating to the student to per-

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mit him, during his course in bacteriology, to observe the lesions produced in various tissues by the different groups and types of microorganisms. During the course in bacteriology it is not the intention to attempt to teach the pathologic lesions of the disease, but rather to make his study more interesting and practical than is possible by utilizing the technic of bacteriology alone. The students who have been instructed in this manner during their course in bacteriology are much better prepared to enter upon their studies in pathology.

The use of the museum for study and instruction entails considerable work and attention upon the part of the instructing staff to properly correlate the museum and its materials with these other studies. It necessitates bringing the museum specimens to the student, not taking the student for instruction to the museum. This means the repeated handling of many specimens and their conveyance to the laboratories desiring their use. A certain number of specimens are injured in this transport, but I am convinced of its great value over the other method which converts the study of museum preparations into a separate course.

To arrange and accommodate a museum which shall serve this purpose, requires much attention on the part of the professor and, furthermore, also requires a cooperation with the clinical departments from whom this material is gained. Specimens, to serve the best purpose, must illustrate the lesion as it is found in life. Improperly prepared specimens which have altered their shape, their size, their color, are of little value for the type of teaching which I am here discussing. It is necessary for us to improve the technic of preservation and mounting so that the specimens are readily available and the student may thoroughly visualize the tissues as they appeared in the individual. There are many sides to the problem of properly presenting such material. Well organized museums are expensive, but when they have once been introduced into the program of instruction they well repay the initial outlay.

A few years ago we recommended a new fluid for the preservation of natural colors of animal tissues. This method, slightly simplified, we have still in use and can speak for the excellent results which it has given.

Upon coming to Brazil and beginning the nucleus of a museum in the Department of Pathology, I continued to make use of the fluid containing chloral hydrate. As I found, however, that chloral hydrate was an extremely expensive product in Brazil, I was forced to review the methods previously in use and to adapt one which would serve for a tropical climate as well as recommend itself for its cheapness.

The preservation of natural colors in tissues involves two processes: (1) the fixation of the tissues and color, and (2) the preservation for final storage. In the past all methods have required two or three separate fluids. The fixation of tissues consists in subjecting them to chemical solutions which will destroy the normal ferments of the tissues, kill the bacteria, and also render the colloids of the tissues insoluble. During fixation there should be very little alteration in the characters of the tissue, so that no distortion is brought about. Furthermore, during this process of fixation the color contained within the tissues should not be destroyed or so seriously altered that it cannot sub-

sequently be restored. In the Kaiserling method the fixation is dependent upon the use of 10 per cent formalin, which produces a good fixation of the tissues, but at the same time reduces the hemoglobin of the blood and of muscle to a brown color. If this reduction has not been carried too far, the color may again in large part be restored by immersing the specimens in alcohol. The danger of producing too great a reduction of the hemoglobin has always been one of the difficulties in this method. In the method which we have previously advocated, we permitted the fixation of the tissues and the fixation of the color to go on simultaneously without necessitating the reduction of the hemoglobin.

The proper fixation of the tissues is the most important stage in the preparation of gross specimens. We have found that good results are much more easily obtained when some care is given to the preparation of the fixing fluid. We have found, for instance, that one may avoid much alteration in the tissue structure if the basis of the fluid consists of an isotonic salt solution. Furthermore, we have found that the tissues at autopsy as well as those removed surgically are prone to become acid within a short time. To prevent the influence of this acid change, we have added a small quantity of sodium bicarbonate to the salt solution. Such solution, without the addition of other substances, will maintain the color and the characters of gross specimens for several days when placed in the ice-chest.

For the actual fixation of the tissues and the red blood cells we have found nothing which is better than formalin. A pure formalin must be used. This has been added in a concentration of from 3 to 5 per cent to the above isotonic solution. It is preferable to use the formalin in a relatively weak concentration for the purpose of penetration. The use of a 10 per cent formalin solution causes a rapid fixation of the outer surface of the specimen which subsequently will not permit the entrance of the solution into the depth of the tissues. An equally complete fixation is gained by a 3 or 5 per cent solution if the specimen is permitted to remain a little longer in the solution, and the weaker solutions have the advantage, furthermore, of not distorting the specimen.

Sodium chloride	8.5
Sodium bicarbonate	5.0
Formalin	30 to 50
Water	1000

The above forms the basis of the fixing fluids. To this then may be added the chemicals for the preservation of the natural colors. When chloral hydrate is used for this purpose, forty grams are added to the above mixture.

In place of chloral hydrate, we have found that carbon monoxide will serve equally well. The specimens are placed in a large jar containing 3000 c.c. of the above mixture. The jar is provided with an inlet near the base and an outlet at the top or in the tightly fitting cover. Ordinary illuminating gas is passed through the fluid after the specimens have been put into it. Usually the gas pressure is insufficient to flow into the bottom of the jar, so that an electric suction pump attached to the outlet, is necessary. The flow of gas is continued for 15 or 20 minutes. This is repeated each morning for 5 to 7 days.

Specimens which are not very fresh and those like spleen, which contain large quantities of blood, frequently discolor the fixing solutions, which then require renewal.

The specimens, before being placed in the fixing solutions, should be reduced to the desired sizes, and one must avoid placing too many specimens into the fixing jar. Best results are obtained when there is at least five times the quantity of fixing fluid, in relation to the quantity of the specimens to be treated.

As in the case with all the types of fixing fluids, good results are difficult to obtain when large masses of tissue are treated. For example it is almost impossible to obtain satisfactory color fixation by treating a whole liver or spleen or even lung. These organs must be cut, and preferably cut in slices.

The use of carbon monoxide for the preservation of color, gives rise to a beautiful bright red color which is quite stable and is not easily reduced by the formalin. The red blood cells do not lake, and within a few days the blood is well set in the blood vessels, capillaries, and tissues. Muscle hemoglobin likewise retains a good color.

As soon as a specimen is well fixed it should be immediately mounted in an air tight jar. For final mounting we are still using the chloral hydrate solution as we have described above. Moreover, we have found that in the preserving fluid the chloral hydrate need not exceed a two per cent solution.

We have in our possession specimens prepared by the gas fixation method, which are now somewhat over a year old. The color is well retained and resembles closely the results we obtain by the chloral hydrate method. The cheapness of the solution and the ease of preparing the specimen by the gas-fixation method recommends its use, particularly in laboratories where large numbers of specimens are constantly treated. One of the common faults committed by museum technicians is to overload the fixing solution with specimens. The above cheap solution can be removed frequently until the fixation is complete.



## VENTRICULAR FIBRILLATION IN MAN WITH TEMPORARY CARDIAC RECOVERY\*

BY FREDERICK A. WILLIUS, M.D., ROCHESTER, MINN.

A STUDY of the exposed dog heart in which ventricular fibrillation is visualized has made possible the recognition of characteristic electrocardiograms.

The waves resulting from conduction of the impulse through the ventricles and from contraction are absent, being replaced by irregular and variable undulations, following each other so closely that no period of cardiac inactivity is evident. The ventricles during fibrillation reveal extremely rapid and irregular incoordinate movements of minute amplitude. The heart at first shrinks, then dilates and no longer possesses the power of expulsion.

It has been generally conceded that ventricular fibrillation in man is incompatible with life although D'Halluin<sup>1</sup> has observed spontaneous recovery in cats, and Nobel and Rothberger<sup>2</sup> have observed it in dogs. Robinson and Bredeck<sup>3</sup> were the first to obtain electrocardiograms in man, showing temporary cardiac recovery from this disorder of mechanism. The observations in this study were made on a patient during a syncopal attack, associated with ventricular fibrillation, thirty-four and a half hours preceding death. Unconsciousness lasted about ten seconds and was accompanied by ventricular fibrillation.

### REPORT OF A CASE

Case A394327, a man, aged forty-four years, was admitted to the Kahler Hospital, October 20, 1922, complaining of marked shortness of breath, swelling of the legs and cough. His mother had died of heart disease. During the Spanish-American war, twenty-four years ago he had had rheumatic fever, but no subsequent recurrent attacks. Following this illness he had no special cardiac complaints except that he was unable to sleep lying on his left side because of the pounding of the heart. He was actively engaged in the practice of law and his daily routine was strenuous. November, 1921, he had an acute illness, said to be influenza, and two months later, complained of palpitation and shortness of breath on effort. At this time several infected teeth were extracted. A month later severe pain developed in the right upper abdomen which persisted for about five weeks. No fever was noted during this time. Two months before examination in the Clinic the shortness of breath had increased, the patient was unable to lie down, his lower extremities swelled, and he coughed severely.

Physical examination revealed a thin man, evidently critically ill. There was marked dyspnea and he was unable to recline. The face was pasty and slightly edematous, and a distinct cyanosis was present. The heart was enlarged, the dullness extending 5.0 cm. to the right and 12.0 cm. to the left of the middle sternal line. The rhythm was irregularly interrupted by premature contractions. There were blowing systolic and diastolic murmurs at the apex, the former being well transmitted into the axilla. The pulmonic second tone

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was accentuated. The pulse rate was 122 each minute. Coarse, moist râles were heard over both lung bases posteriorly. The liver was not palpable. There was slight edema of the feet. The systolic blood pressure was 98, the diastolic 68. The urine contained a moderate amount of albumin and an occasional hyalin cast. Roentgenographic examination of the chest showed the heart to be markedly enlarged and there was infiltration of the lower right lobe which was thought to be due to congestion. The temperature was normal, or subnormal, throughout the illness.

#### SUBSEQUENT COURSE AND TREATMENT

Twenty-two c.c. of Minnesota leaf tincture of digitalis was given in four days. Fluids were restricted to 800 c.c. in twenty-four hours and the patient placed on a light protein and salt-free diet. Digitalis was given intermittently.

The patient showed no response to rest and treatment; all the symptoms became exaggerated and were accompanied by marked restlessness, requiring frequent opiates.

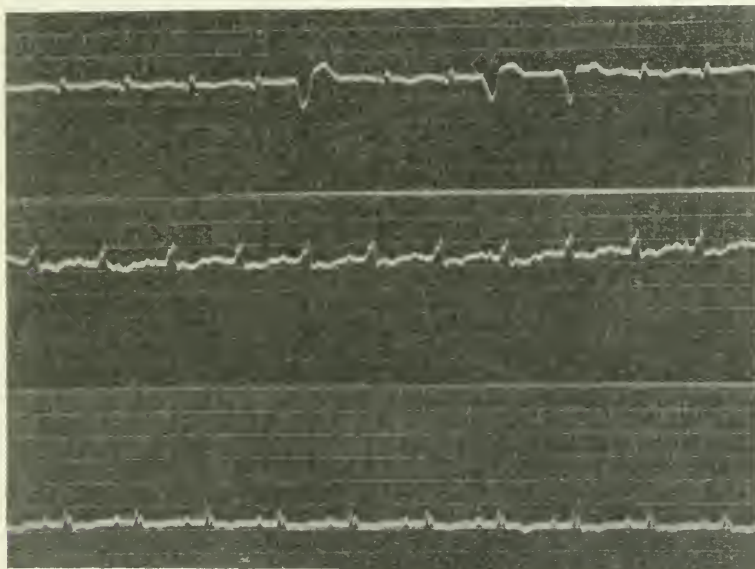


Fig. 1.

November 2 drowsiness was noted and while no appreciable qualitative change in the urine was evident, the amount was less, only 200 c.c. having been excreted the previous day. The blood urea was found to be 68 mg. and the urea nitrogen 31.7 mg. for each 100 c.c.

November 6 the drowsiness was more marked and the urea had increased to 144 mg. and the urea nitrogen to 67 mg. for each 100 c.c. On this day expectoration of bright red blood occurred, but there was no complaint of chest pain. During the course of electrocardiographic examination the patient suddenly became unconscious, there was no perceptible pulse at the wrist, cyanosis became quite profound and death seemed imminent. This attack lasted about ten seconds, after which the patient was much weaker,

and death occurred thirty-four and a half hours later. There was no repetition of syncope.

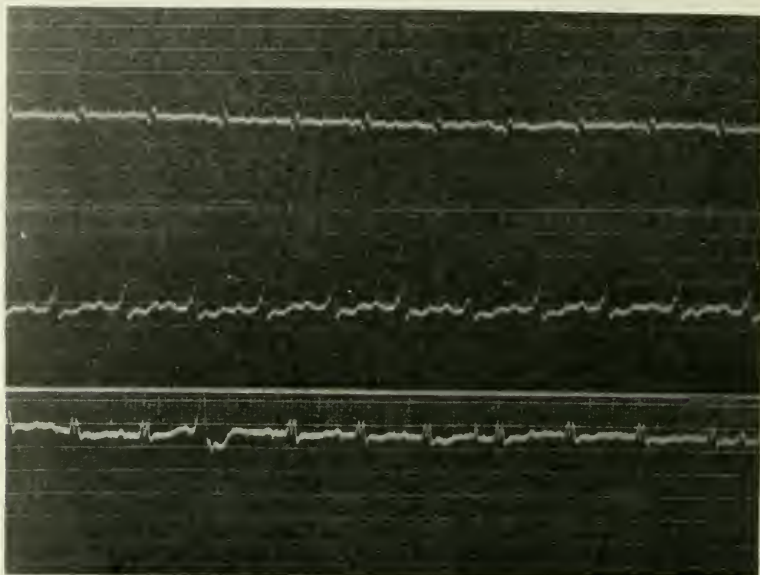


Fig. 2.

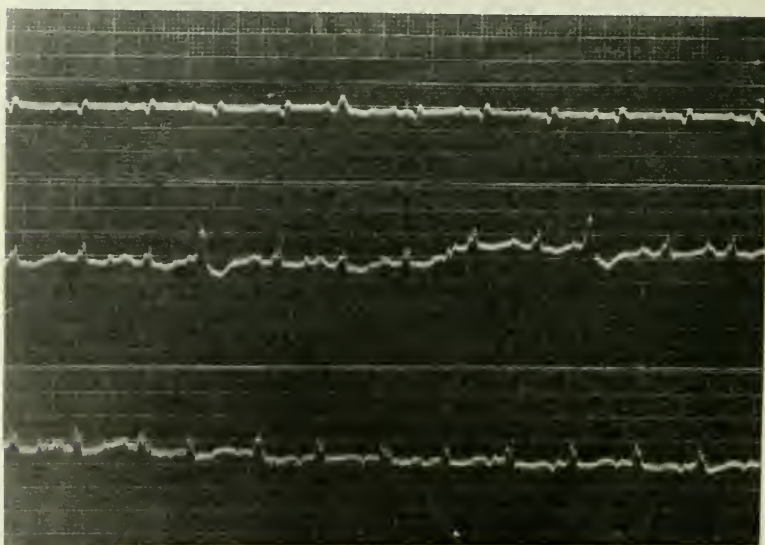


Fig. 3.

#### ELECTROCARDIOGRAMS

The first electrocardiogram (Fig. 1), taken the second day after admission, showed a rate of 100, and premature contractions of ventricular origin. The QRS complexes are aberrant in all derivations, being definitely notched. The T wave is negative in all derivations. No other abnormalities exist.

The second record (Fig. 2), obtained November 1, shows no appreciable change.

The third record (Fig. 3) was taken November 3 and besides the features

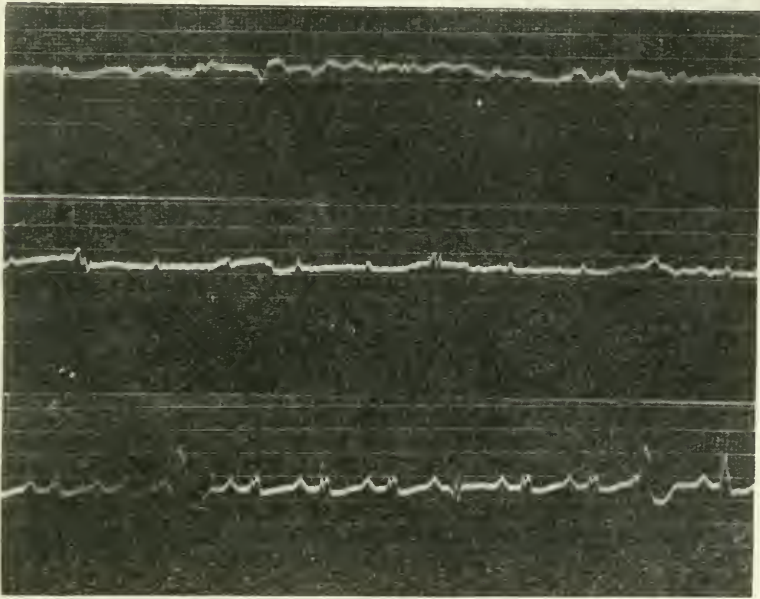


Fig. 4.

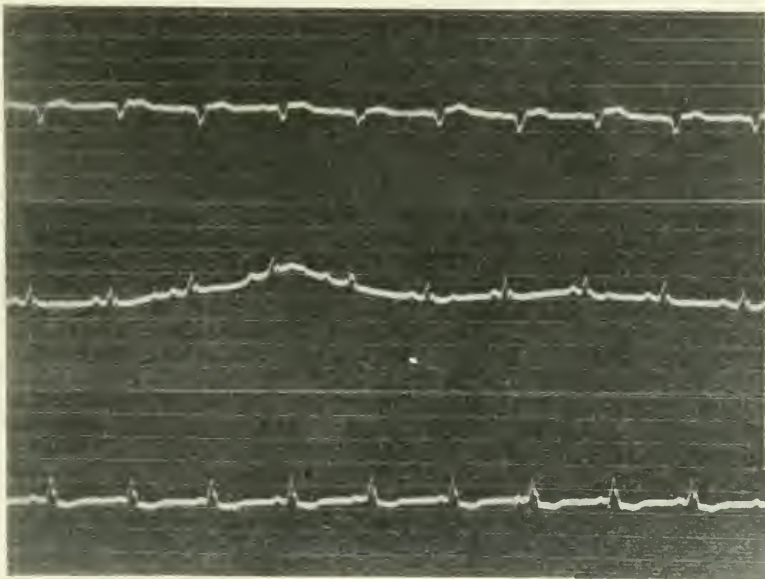


Fig. 5.

noted in the previous electrocardiograms, notching of the P wave in Derivation II and negativity of the P wave in Derivation III are present.

The fourth record (Fig. 4), taken November 6, shows ventricular fibrillation in Derivation I and was coincident with the syncopal attack previously



described. By the time that Derivation II was recorded the preexisting cardiac mechanism was reestablished. Unfortunately the transitional record was not obtained.

The last record (Fig. 5) was obtained November 7, eleven and a half hours before death. The QRS complexes are aberrant in all derivations and directed downward in Derivation I.

#### NECROPSY

The heart was enormously enlarged, principally as a result of dilatation. The weight of the organ was 610 grams. There was extensive myocarditis, practically all muscle fibers being involved. A low grade chronic endocarditis of the aortic and mitral valves was present. There was a mural thrombosis of the right auricular appendage with multiple infarcts of both lungs. The liver showed chronic passive congestion with marked atrophy. The spleen, kidneys, intestines and lungs were greatly congested. The kidneys were otherwise normal. A terminal bronchopneumonia was also present.

Histologic studies of the myocardium revealed, besides the extensive muscle degeneration, an occasional Aschoff body.

The extensive myocardial damage found at necropsy is a very tangible explanation of the ventricular fibrillation. The extreme dilatation of both ventricles further favored the development of this abnormal mechanism.

#### SUMMARY

A case is presented in which the patient showed progressive cardiac failure. During the syncope attack fibrillation of the ventricles occurred. The patient lived thirty-four and a half hours after the attack. This case is the second instance of temporary cardiac recovery in man observed following ventricular fibrillation.

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## DUODENECTOMY, REPORT OF EXPERIMENT FOUR YEARS AFTER OPERATION\*

BY FRANK C. MANN, M.D., AND KYOICHI KAWAMURA, M.D., ROCHESTER, MINN.

IN two previous publications we have described the technic for the removal of the duodenum and recorded the general results following such operation on several species of animals. We shall report here one experiment in which the animal (dog) was in good condition four years after duodenectomy.

*Protocol.*—December 11, 1918. Experiment 892. Dog C878, a male mongrel shepherd, weighing 20.7 kg., was placed under ether anesthesia and the duodenum removed at a single operation by the method previously described. The animal recovered from the operation and was kept under observation for four years. During this time the animal remained in excellent condition. It was weighed at intervals, its weight usually fluctuating between 19 and 21 kg. During the early part of the winter of 1920, it became slightly thin, its weight decreasing to 16.4 kg., the lowest recorded during the four years. Since 1920 it had not weighed less than 19 kg. The animal was always active, his appetite good and general condition excellent. It appeared perfectly normal, and no effects of the operation were observed. It was photographed several times, and the pictures compared. No difference was discernible.

December, 1922, four years after the operation, the animal weighed 20.5 kg. and was in excellent condition. It was killed by over-etherization, and necropsy was performed immediately. The animal was fat and the muscles were well developed. The site of abdominal incision was marked by a faint, hardly discernible scar. In the abdominal cavity the main evidence of a previous operation was a few adhesions of omentum to the site of the anastomosis of the jejunum to the stomach. The pancreas was small, weighing 12 gm., hard on palpation, but normal in general appearance. Both ducts were dilated, the major measuring 3 mm. in diameter. The liver was normal in color and size, weighing 500 gm. The organ cut with more than normal resistance and gave other evidence of increased connective tissue formation. The common bile duct measured 6 mm. in diameter and the hepatic ducts were also dilated. The gall bladder was shrunken to about one-third its normal size. The walls were thickened and pale. On palpation the gall bladder felt hard; it was opened and the contents were found to be white, thick and viscid with almost no odor. The mucosa was thickened and pale. The gastric and intestinal mucosae were normal in appearance. All other organs were grossly normal.

Microscopic examination revealed that all organs were normal except the liver, pancreas and gall bladder. The liver had a slightly increased

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amount of perilobular connective tissue with round-cell infiltration in some areas. The walls of the bile ducts were thickened. On the whole, the organ was practically normal. The pancreas was normal except for an increase in the connective tissue around the ducts. The gall bladder showed the greatest changes. The walls were thickened with an increased connective tissue, and there was a large amount of lymphocytic infiltration throughout their entire thickness, particularly marked just under the mucosa. The mucosa was thickened and the cells enlarged.

#### SUMMARY

The duodenum was removed from a dog and the continuity of the gastrointestinal tract maintained by an end-to-end anastomosis of jejunum to stomach. The first portion of the jejunum thus assumed the position normally occupied by the duodenum. The common bile duct and pancreatic ducts were transplanted into this transposed portion of the jejunum at approximately the same distance from the pylorus and from each other as they occurred normally.

The experiment is of interest, (1) in showing the effect of removal of the duodenum; and (2) the effect of transplantation of the common bile and pancreatic ducts. The animal remained in perfect health and maintained its normal weight for four years following removal of the duodenum, and there is no reason to believe that the duodenectomy would ever have affected the health of the animal, had it been allowed to live longer. The experiment therefore definitely proves that in the dog the duodenum is not essential to life or good health, and that whatever function it may have can be compensated for by the remainder of the intestinal tract. It is also shown that the transplantation of the bile and pancreatic ducts can be carried out successfully, so that the respective glands will remain practically normal for a long time.

The conclusion made by Dragstedt et al. Grey, Moorhead and Landes, and by us, regarding duodenectomy, based on relatively short periods of observation, is corroborated by this experiment based on a long period.

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## CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

BY A. LEVINSON, M.D., CHICAGO, ILL.

(Continued from page 439.)

### BLOOD CHEMISTRY

THE chemical determinations on blood of clinical value are: sugar, non-protein nitrogen, urea nitrogen, uric acid, creatinine, cholesterol, chlorides calcium and phosphorus. Of these, the first five mentioned are most important.

The most commonly employed system of blood analysis is that of Folin and Wu. Because of the rapidity of the methods and the small amount of blood needed in this system of analysis, it seems preferable to other methods. I shall therefore describe it here as used with slight modification at the Michael Reese Hospital chemical laboratories. For detailed accounts one should refer to the original work of Folin and Wu. It should be remembered, however, that because of the small amount of material needed and because of the personal factor entering into the reading of colorimeters, a slight inaccuracy in the method may mean a large percentage of error in the final results.

**REMOVAL OF PROTEINS.**—In determining sugar, nonprotein nitrogen, urea, uric acid and creatinine in the blood, the protein must first be coagulated. The following method is used: To a measured quantity of oxalated blood, (2 to 8 c.c. depending on the determinations desired) are added seven volumes of water, one volume of 10 per cent sodium tungstate, and one volume of 2.3 normal sulphuric acid, the latter to be added slowly from a burette while shaking the flask. The mixture is allowed to stand for at least five minutes, to coagulate the proteins. The color of the precipitate should now change from red to dark brown or chocolate. If the change in color does not occur, the blood has not been coagulated completely, most likely because too much oxalate has been used in obtaining the specimen. In such cases, 10 per cent sulphuric acid should be added drop by drop and the coagulum shaken after each drop until the color changes to chocolate. The mixture is now filtered through a large, dry filter. The clear, colorless filtrate is used for the various examinations. If the filtrate is to stand 24 hours or longer before examination, a drop or two of toluol should be added.

**DETERMINATION OF BLOOD SUGAR.**—Into a Folin sugar tube (pyrex), graduated at 25 c.c. (Fig. 14) should be introduced 2 c.c. of blood filtrate and 2 c.c. of alkaline copper tartrate solution.<sup>1</sup> Into another tube should be introduced

<sup>1</sup>*Alkaline Copper Tartrate Solution.* Forty grams of anhydrous sodium carbonate is dissolved in about 400 c.c. of water. This is transferred to a one liter volumetric flask. Now 7.5 grams of tartaric acid are added. When dissolved, 4.5 grams of crystallized copper sulphate are added, mixed and made up to the mark.



2 c.c. of a standard sugar solution<sup>2</sup> containing 0.1 mg. of glucose per c.c., and 2 c.c. of alkaline copper tartrate. The standard and unknown are heated together in boiling water for six minutes, after which they are placed in cold water for two minutes. To each tube is then added 2 c.c. of molybdate phosphate solution<sup>3</sup> and water is added after two minutes to the 25 c.c. mark. The solutions are mixed well and after standing for at least five minutes the unknown is read against the standard in the colorimeter setting the standard at 20.

The sugar is calculated from the formula:

$$\frac{20 \times 100}{\text{Reading}} = \text{mgs. of sugar per 100 c.c. of blood.}$$

The above method is simpler than the older blood sugar methods. The blue color of the standard is a great advantage over the older methods, where the matching of yellow colors is required.

**SUGAR TOLERANCE.**—In addition to the determination of sugar on one specimen of blood, the sugar tolerance test may be employed for the detection of diabetes and of endocrine disturbances. The sugar tolerance test consists of the determination of the blood sugar at repeated intervals, namely, before the ingestion of sugar,  $\frac{1}{2}$  hour, 1 hour, 2 hours, 3 hours and 4 hours after the ingestion of sugar.

One and one-half gm. of glucose is given to each kilogram of body weight. The sugar is given in water or in lemon juice on an empty stomach. In normal individuals, the blood sugar rises from normal, (80 to 120 mg. per 100 c.c.) to 130-180 mg. one hour after the ingestion of the sugar, and returns to normal within 2 or 2 $\frac{1}{2}$  hours. No sugar appears in the urine.

In diabetes the blood sugar rises to 300 mg. or even higher in  $\frac{1}{2}$  to 2 hours and it takes several hours before it returns to normal.

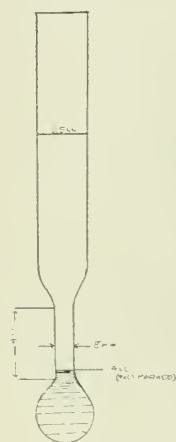


Fig. 14.  
Folin sugar tube.

In endocrine disturbances, the rise in the sugar curve is even slower than normal, the patient having an increased tolerance for sugar.

**DETERMINATION OF NONPROTEIN NITROGEN.**—Five c.c. of blood filtrate is introduced into a pyrex digestion tube, which is calibrated at 35 c.c. and 50 c.c., and 1 c.c. of acid digestion mixture<sup>4</sup> is added. A glass bead is put in to prevent bumping. The mixture is now heated rapidly over a microburner until

<sup>2</sup>*Standard Sugar Solution.* A stock solution is made by dissolving 1 gm. of pure glucose in 100 c.c. of benzoic acid solution (2.5 gm. benzoic acid in 1 liter of boiling water, allowed to cool). Before using, it is diluted with 0.3 per cent benzoic acid one to one hundred. For each blood determination 1 c.c. of the stock solution is introduced into a 100 c.c. volumetric flask, and benzoic acid is added to the mark. Two c.c. of this standard, containing 0.1 mg. of glucose per cubic centimeter, is used.

<sup>3</sup>*Phosphomolybdic Acid Solution.* Thirty-five grams of molybdic acid and 5 grams of sodium tungstate are placed in a liter beaker, 200 c.c. of 10 per cent sodium hydroxide and 200 c.c. of water are added. This is boiled vigorously for twenty to forty minutes to remove nearly all of the ammonia present in the molybdic acid, cooled, diluted to about 350 c.c., 125 of 85 per cent phosphoric acid added and diluted to 500 c.c.

<sup>4</sup>*Acid Digestion Mixture.* Three hundred c.c. of 85 per cent phosphoric acid is mixed with 100 c.c. of concentrated sulphuric acid (free from ammonia). This is transferred to a tall cylinder, covered well to prevent absorption ammonia, and set aside for at least a week to permit sedimentation of calcium sulphate. To 100 c.c. of the clear acid, 100 c.c. of water and 10 c.c. of 6 per cent copper sulphate solution is added.

the water is boiled off. When white fumes appear the tube is covered with a small watch crystal and heated slowly at the boiling point until the solution assumes a pale green color, or at least until it fumes two minutes. The contents are allowed to cool for 60 to 90 seconds and water is then added, drop by drop at first and then to about 30 c.c. While cooling, the standard should be prepared as follows:

Into a 100 c.c. volumetric flask is introduced 3 c.c. of an ammonium sulphate solution containing 0.3 mg. of nitrogen<sup>5</sup>, 2 c.c. of the acid digestion mixture, and water to about 50 c.c.

Fifteen c.c. of Nessler's solution<sup>6</sup> are now added slowly, with constant shaking, to the unknown and 30 c.c. to the standard. The blood filtrate and standard should be nesslerized about the same time. Distilled water is added to the mark and the contents of the flasks mixed thoroughly.

If the blood filtrate solution is cloudy, due to the suspensions of particles of silica from the glass, it should be centrifuged. The solution is now read in the colorimeter against the standard solution of ammonium sulphate setting the standard at 20.

The nitrogen content is calculated from the formula:

$$\frac{20 \times 30}{\text{Reading}} = \text{mg. of nitrogen in 100 c.c. of blood.}$$

**DETERMINATION UREA NITROGEN.**—Into a pyrex nitrogen tube are introduced 5 c.c. of the blood filtrate, two drops of pyrophosphate buffer mixture<sup>7</sup> and 1 c.c. of urease solution.<sup>8</sup> The tube is placed in water at 40° to 55° C. and allowed to stand for 5 minutes or left at room temperature for 15 minutes. Two c.c. of saturated borax solution are then added to render the solution alkaline. Five drops of paraffin oil and a glass bead are also added to prevent foaming. The mixture is distilled into a pyrex test tube graduated at 25 c.c. containing 2 c.c. of twentieth normal hydrochloric acid, the distillation consuming 5 minutes. The distillate is cooled under the tap. A standard solution is made up by placing in a 100 c.c. volumetric flask, 3 c.c. of the standard ammonium sulphate solution and about 60 c.c. of water. Standard and unknown are then nesslerized, the standard receiving 10 c.c. and the unknown 2.5 c.c. The standard is then made up to the 100 c.c. mark and the unknown to the 25 c.c. mark.

<sup>5</sup>*Standard Solution* of ammonium sulphate. Specially purified ammonium sulphate, 0.4716 g., is dissolved in 1000 c.c. of distilled water. Ten c.c. of this solution contains 1 mg. of nitrogen. For blood standard 3 c.c. is used. Toluol is added as a preservative.

<sup>6</sup>*Nessler's Solution.* One hundred gm. of mercuric iodide and 70 gm. of potassium iodide are introduced into a liter volumetric flask and 400 c.c. of water added. One hundred gm. of sodium hydroxide dissolved in 500 c.c. of water and cooled thoroughly is now added to the mixture in the flask, and water is added to make up a liter. The small amount of red precipitate should be allowed to settle at the bottom and the supernatant fluid should be used.

<sup>7</sup>*Buffer mixture.* Seventeen and two-tenths gm. of monosodium phosphate and 44.8 gm. of crystallized disodium phosphate are dissolved in 200 c.c. of warm distilled water. The solution is cooled and diluted to 250 c.c. One or two drops of Toluol is added as a preservative.

<sup>8</sup>*Urease Solution.* About 3 gm. of permutit powder are washed with 2 per cent acetic acid, then twice with water. Five grams of Jack bean meal and 100 c.c. of 15 per cent alcohol (16 c.c. of ordinary alcohol and 84 c.c. H<sub>2</sub>O) are added. The flask is stoppered and shaken gently but continuously for 15 minutes after which it is filtered through a large dry filter. It should be kept in a cold place. The solution then keeps about a week at room temperature and four to six weeks in an ice box.

Urea nitrogen is calculated from the formula :

$$\frac{St \times 15}{R} = \text{mg. per 100 c.c. blood.}$$

**DETERMINATION CREATININE.**—Ten c.c. of the protein-free blood filtrate are measured into a large test tube. Into another tube are introduced 5 c.c. of a standard creatinine solution<sup>9</sup> (containing 0.03 mg. of creatinine), and 15 c.c. of water. A fresh alkaline picrate solution is prepared by mixing 15 c.c. of saturated freshly recrystallized picric acid and 3 c.c. of 10 per cent sodium hydroxide purified by alcohol. This solution is then added to standard and unknown, 10 c.c. to the former and 5 c.c. to the latter. Both are allowed to stand for 10 minutes before reading in the colorimeter, the standard being set at 20.

Calculate from the formula :

$$\frac{20 \times 1.5}{\text{Reading}} = \text{mg. per 100 c.c. blood.}$$

**DETERMINATION OF URIC ACID.**—Into a test tube graduated at 25 c.c. is measured 5 c.c. of blood filtrate. Into another such tube is measured 5 c.c. of standard uric acid solution containing 0.02 mg. of uric acid.<sup>10</sup> To each is added 2 c.c. of water, 2 to 3 drops of 20 per cent lithium sulphate solution<sup>11</sup> and 2 c.c. of sodium cyanide solution.<sup>12</sup> One c.c. of uric acid reagent<sup>13</sup> is added to each, the solutions are mixed and allowed to stand for two minutes. They are then placed in boiling water for 80 seconds, cooled in running water and made up to the mark with water, mixed and read against the standard which is set at 20.

$$\frac{20 \times 4}{\text{Reading}} = \text{mg. of uric acid per 100 c.c. of blood.}$$

**DETERMINATION OF CHLORIDES.**—Into a small Erlenmeyer flask is measured 0.115 c.c. of blood plasma and 1.6 c.c. of N/100 silver nitrate solution.<sup>14</sup> To

<sup>9</sup>*Creatinine Standard Solution.* A stock solution containing 1 mg. of creatinine per c.c. (used also for determination of creatinine in urine) is made up by dissolving 1.61 gm. of creatinine zinc chloride in 1 liter of N/10 HCl acid. Six c.c. of this solution are measured into a liter volumetric flask, 10 c.c. of normal HCl added and water to the mark. Toluene is used as a preservative.

<sup>10</sup>*Standard Uric Acid Solution.* A stock solution is made up as follows: Exactly one gram of uric acid is transferred to a funnel on a 300 c.c. flask. From 0.45 to 0.5 gm. of lithium carbonate is placed in a 300 c.c. beaker, 150 c.c. of water is added, and the mixture heated to 60° C. with constant stirring to dissolve the salt. The uric acid is then rinsed into the flask and with shaking dissolves practically at once. As soon as a clear solution is obtained, it is cooled under running water with shaking, and transferred to a liter volumetric flask, rinsed and diluted to 400 to 500 c.c. Twenty-five c.c. of 40 per cent formaldehyde are added and after shaking, 3 c.c. of glacial acetic acid. The solution is shaken to remove most of the carbonic acid, diluted to volume and mixed. It should be tightly stoppered and kept in the dark.

For use it is diluted 1:250 as follows: 1 c.c. is transferred to a 250 c.c. volumetric flask half full with water. Ten c.c. of 2.3 NH<sub>2</sub> SO<sub>4</sub> and 1 c.c. of 40 per cent formaldehyde are added, diluted to the mark and mixed.

<sup>11</sup>*Lithium sulphate solution.* Twenty gm. of powdered lithium sulphate are dissolved in about 80 c.c. of cold water. It is diluted to 100 c.c. and filtered.

<sup>12</sup>*Sodium cyanide solution* (approximately 15 per cent in N/10 NaOH). One hundred to 150 gm. of cyanide are transferred to a large beaker and 6.7 c.c. of N/10 NaOH is added for each gram taken, stirring occasionally until dissolved.

<sup>13</sup>*Uric acid reagent.* (a) Fifty c.c. of 85 per cent phosphoric acid and 160 c.c. of water are transferred to a 500 c.c. Florence flask. This is heated nearly to boiling and then 100 gm. of sodium tungstate is added. This is boiled gently but continuously for one hour over a micro-burner using a 200 c.c. flask filled with cold water on a funnel as a condenser.

(b) In a liter beaker is placed 25 gm. of lithium carbonate. Fifty c.c. of phosphoric acid and 200 c.c. of water are added carefully. The carbon dioxide gas is boiled off and the mixture is cooled. Solutions (a) and (b) are mixed and diluted to 1 liter.

<sup>14</sup>*N/100 silver nitrate solution.* 1.699 gm. per liter.

this is added 10 drops of chloride-free concentrated nitric acid. The mixture is then warmed carefully and saturated potassium permanganate solution is added drop by drop until the color it produces remains. The solution is boiled carefully for five minutes and then dextrose is added drop by drop until the color disappears. When cool, the silver chloride should lie on the bottom in little lumps and the supernatant liquid should be colorless. The excess silver nitrate is then titrated with N/100 ammonium thiocyanate solution<sup>15</sup> four to five drops of saturated ferric ammonium sulphate solution being used as indicator.

Chlorides are calculated from the formula:

$$\frac{\text{c.e. of silver nitrate} - \text{c.e. of thiocyanate used}}{\text{volume of blood used (0.115 c.e.)}} \times 0.0585 =$$

gm. of NaCl per 100 c.e. of blood.

DETERMINATION OF CHOLESTEROL.—With constant shaking 3 c.e. of blood are run slowly into a 100 c.e. volumetric flask containing about 80 c.e. of redistilled alcohol and ether (3:1). The mixture is heated to boiling on a water-bath, with occasional shaking. It is then cooled under the tap, made up to 100 c.e. with the alcohol-ether mixture, and filtered through a dry filter.

Ten c.e. of this extract are measured into a small beaker and evaporated just to dryness on a water-bath. The cholesterol is extracted from the dry residue by boiling out three or four times with 2 to 3 c.e. of chloroform and decanting into a beaker. The combined extracts are evaporated to less than 5 c.e. and transferred to a glass stoppered 10 c.e. graduated cylinder. The volume is made up to 5 c.e. with chloroform.

Into another graduated cylinder are introduced 5 c.e. of a standard cholesterol solution<sup>16</sup> (containing 0.5 mg. of cholesterol). To both standard and unknown are added 2 c.e. of acetic anhydride and, drop by drop, 0.1 c.e. of concentrated sulphuric acid. The solutions are carefully mixed and set in the dark for 15 minutes after which they are read in the colorimeter. The standard is placed at 15.

Cholesterol is calculated from the formula:

$$\frac{25 \times 100}{\text{Reading}} = \text{mg. per 100 c.e. blood.}$$

INTERPRETATION OF BLOOD CHEMISTRY.—Blood sugar in children is approximately the same as that of adults, namely, 0.08 per cent to 0.12 per cent. These figures vary with the intake of food, being higher after a meal. A marked increase in blood sugar without previous ingestion of food indicates diabetes. This is of greater significance than the reduction test in urine, as the latter might not always indicate the presence of a sugar, for as is well known, salicylates and other drugs might cause a reduction of the urine by copper sulphate. There is also the condition known as renal diabetes occur-

<sup>15</sup>N/100 ammonium thiocyanate solution. 0.761 gm. per liter.

<sup>16</sup>Cholesterol Standard. A stock solution of 0.2 gm. of cholesterol in 200 c.e. chloroform is diluted 1:10 with chloroform giving the standard solution for use.



ring in nondiabetic individuals. In this condition there is a glycosuria without any increase of blood sugar.

Nonprotein nitrogen of blood in children usually runs between 25 to 40 mg. per 100 c.c. of blood. This is increased in uremia and in acute and chronic nephritis with nitrogen retention. Schloss found a marked increase in nonprotein nitrogen in cases of intestinal intoxication.

Urea nitrogen varies in normal infants and children between 12 to 20 mg. per 100 c.c. of blood. In other words, it constitutes about 50 per cent of the nonprotein nitrogen. An increase in urea may be the first indication of nitrogen retention.

Uric acid varies between 1-4 mg. per 100 c.c. of blood. Uric acid often is the first nonprotein nitrogenous product to be retained in the blood. Creatinine has a very important prognostic significance. An increase of creatinine usually means a bad prognosis. An increase in other nonprotein nitrogen constituents may not necessarily mean a bad prognosis.

Cholesterol varies between 150 to 175 mg. per 100 c.c. of blood. It is increased in diabetes and occasionally also in nephritis.

Blood chlorides vary between 500 to 600 mg. per 100 c.c. of blood, figured as sodium chloride. They are often increased in nephritis with edema and may be decreased proportionally in the urine.

Acetone and diacetic acid are normally present only in small traces, while in uremia and diabetes they may be present in large quantities.

TABLE VI

CHEMICAL CONSTITUENTS OF BLOOD IN MG. PER 100 C.C.

Nonprotein Nitrogen .....	25.00- 40.00
Urea Nitrogen .....	12.00- 20.00
Uric Acid .....	1.00- 4.00
Creatinine .....	0.9- 2.0
Sugar .....	80.00-120.00
Chlorides .....	500.00-600.00
Cholesterol .....	150.00-175.00
Calcium .....	10.0 - 11.5
Phosphorus (Inorganic) .....	4.8 - 6.8

REACTION OF BLOOD.—Normally, the blood is slightly alkaline. The reaction is usually expressed in terms of pH, blood having a pH of 7.4 to 7.6 compared to distilled water which is neutral or of pH 7.0. Although the blood is only slightly alkaline so far as its reaction is concerned, it has the power of neutralizing quite a large amount of strong acid before its reaction is changed. This power of the blood is due to the presence in the blood of sodium bicarbonate, disodium hydrogen phosphate and alkaline salts of proteins, which are known as "buffer substances." They constitute the so-called alkaline reserve of the blood. In diabetes, some alimentary disturbances, and some febrile conditions, the alkaline reserve of the blood is diminished. The condition is then spoken of as acidosis, although the blood is not really acid in reaction or life would be impossible.

The degree of acidosis may be determined by the examination of either the urine for increase in ammonia, presence of acetone and diacetic acid, the



alveolar air for carbon dioxide tension and above all, the blood for alkaline reserve or the H-ion concentration.

**ALKALI RESERVE.**—Normally the alkali reserve varies between 40 to 63 per cent of  $\text{CO}_2$  at "O" temperature and 750 barometric pressure. The alkali reserve is expressed in terms of R<sub>p</sub>H.

*Method.*—Either the Van Slyke and Cullen apparatus or the Marriott colorimetric method may be used. The microapparatus of Van Slyke is more applicable to children's work than the macroapparatus, as only 0.2 c.c. is required in the former apparatus. The Marriott method is more frequently used in clinical work, because the colorimeter is portable and readily obtainable.

The Marriott method consists of dialyzing whole blood, or blood serum, against a salt-indicator solution having a pH of 7.0, and comparing the re-

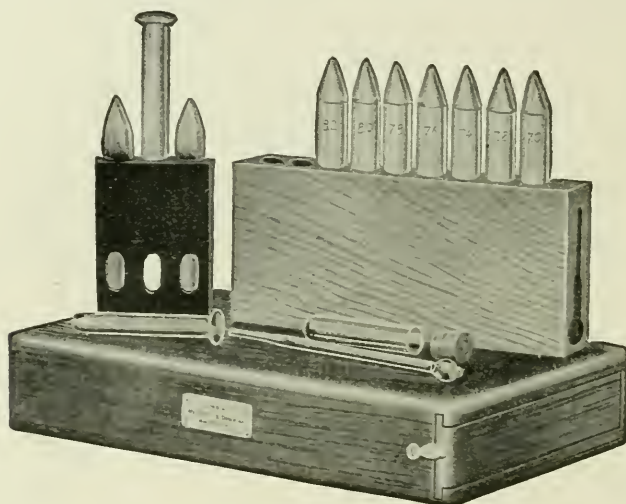


Fig. 15.—Alkali reserve tubes.

sulting color change with standard indicator tubes. A set of these standard tubes may be obtained from physicians' supply houses.

*Preparation of Sacks.*—Sacks of celloidin or Anthony's negative cotton, are used for dialysis. They are made as follows: One ounce of celloidin is dissolved in 500 c.c. of a mixture of equal parts of alcohol and ether. The solution is allowed to stand for a week to permit impurities to settle.

A test tube about 50 mm. long and 6 mm. in diameter is filled with the celloidin solution, and slowly poured out with a rotary motion. This leaves an even coating on the inner surface of the tube. After draining and drying for ten minutes the tube is filled with cold water, the upper edge of the celloidin sack is loosened and water poured between it and the tube, and the sack removed from the test tube. Several of these sacks may be made at one time and preserved by immersing them in water.

The salt indicator solution is made as follows: Eight gm. of chemically pure sodium chloride is dissolved in distilled water, 220 c.c. of 0.01 per cent

solution of phenolsulphonephthalein is added and the whole made up to one liter with distilled water. Jena glassware is used.

This solution should have a pH of 7.0, and if it does not prove so on comparison with the standard indicator tubes, should be titrated to this point with weak alkali or acid.

*Procedure.*—The celloidin sacks to be used are immersed in some of the salt-indicator solution. A few c.c. of blood are withdrawn with a needle from one of the patient's veins, care being taken that the blood does not hemolyze. The blood is centrifuged at once to separate the serum. One-half c.c. of the serum is measured into one of the celloidin sacks, and the sack suspended in a larger test tube containing 2 c.c. of a salt-indicator solution. The fluid level must be at least as high on the outside as on the inside of the sack. At the end of 7 minutes the sack is removed. The dialysate is transferred to a tube of the same diameter as the standard indicator tubes. A rapid cur-

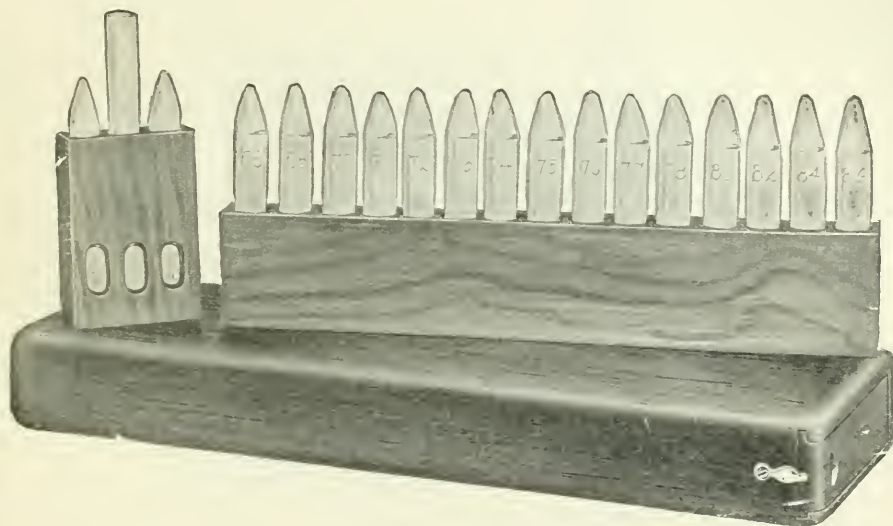


Fig. 16.—Hydrogen-ion concentration tubes.

rent of air is blown through the solution to remove the carbon dioxide. An atomizer bulb and a small glass tube is satisfactory for this purpose. After three minutes the color of the solution is compared with that of the standard tubes. The readings are given on the tubes in terms of RpH (Fig. 15). The normal values are 8.4 to 8.55 corresponding to an alveolar  $\text{CO}_2$  tension of 35 to 45 mm. Values from 8.0 to 8.3, corresponding to an alveolar  $\text{CO}_2$  of 28 to 35 mm, indicate moderate acidosis. Values below 7.7 indicate immediate danger of coma. The above values hold good for adults and older children. Infants give lower readings. They may show an RpH of 8.3 and yet have no evidence of acidosis.

The hydrogen-ion concentration outfit (Fig. 16) is sometimes used in clinical work to determine an advanced state of acidosis.

The following table from Palmer and Van Slyke, may be utilized clinically in the treatment of acidosis:



TABLE VIII

WEIGHT OF INDIVIDUAL		SODIUM BICARBONATE NECESSARY TO RAISE PLASMA $\text{CO}_2$ I VOLUME PER CENT
kg.	lb.	gm.
19	42	0.5
38	84	1.0
57	126	1.5
76	168	2.0
95	210	2.5

**ALVEOLAR AIR TENSION.**—Normally the alveolar air tension varies between 40 and 70 millimeters of mercury. In acidosis less  $\text{CO}_2$  is expired by the lungs and the alveolar air is therefore lowered. The determination of alveolar air is thus one of the methods of determining the presence of acidosis.

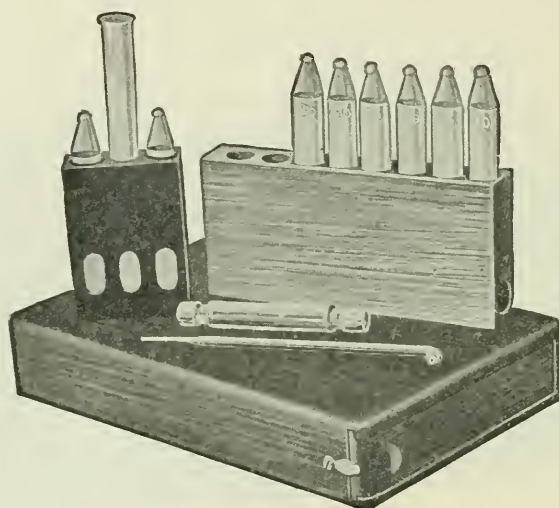


Fig. 17.—Alveolar air tubes.

**Technic.**—A set of standard color tubes is used. The tubes may be obtained on the market.

A standard bicarbonate solution is made either by weighing out 0.530 grams of desiccated sodium carbonate, or by measuring accurately 100 c.c. of tenth normal sodium hydroxide into a liter flask. Two hundred c.c. of 0.01 per cent phenolsuphonephthalein are added and the whole made up to the mark with distilled water. The flask containing the solution should be kept corked and paraffined.

A rubber bag of about 1500 c.c. capacity is used to collect the alveolar air. A basket ball bladder answers the purpose. For infants a bag of 500 c.c. capacity and a small rubber funnel to take the place of a mask are used. The bag is half filled with air with an atomizer bulb, and the patient breathes back and forth into the bag, 4 times in 20 seconds. The bag is then closed by a pinchcock.

Two or three c.c. of the standard bicarbonate solution are now placed

in a small test tube similar to those containing the standard color solutions. Air from the bag is then blown through the solution by a glass tube drawn out to a small bore, until the solution is saturated. When no further color change takes place the saturation is complete. The tube is stoppered and the color at once compared with the standard color tubes. The numbers on the tubes (Fig. 17) give the carbon dioxide tension, in mm. The alkali reserve of the blood may be obtained by multiplying the alveolar air tension by 0.7.

#### BLOOD TRANSFUSION.—

*Indications.*—1. Loss of blood, such as in hemorrhage of the newly born, or trauma.

2. Inanition, such as in prolonged alimentary disturbances.
3. Severe sepsis.
4. Jaundice.
5. Various anemias of childhood.
6. Hemophilia.

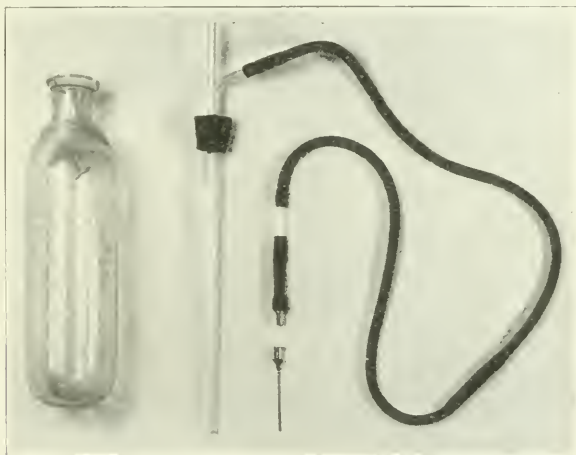


Fig. 18.—Bottle, rubber tubing, metal tip and needle (detached).

*Implements Necessary.*—The number of vessels and instruments necessary depends on the method to be used for the introduction of the blood into the child. If the blood is to be given subcutaneously, only 2 needles, 18 to 20 gauge, 3 Luer syringes and a tourniquet are necessary. If the blood is to be given intravenously or into the sinus the following vessels and solutions are necessary:

- (a) One venipuncture needle, for the removal of blood from the donor.
- (b) One needle for the introduction of blood into the child. The size of the needle depends on the route to be used for the introduction of the blood. If it is to be introduced into a vein on the child's arm or leg, or into the external jugular vein, a regular venipuncture needle is to be used. If it is to be introduced into the longitudinal sinus, a short sinus needle is to be used.
- (c) Two Luer syringes of 20 c.c. capacity, to be used for the removal of the blood.

(d) Two per cent sterile solution of sodium citrate. Ten c.c. of citrate to be used for each 100 c.c. of blood.

(e) One or 2 medicine glasses as containers of the citrate.

(f) One wide mouth flask or jar into which the citrate and the blood is to be emptied.

(g) One funnel containing several layers of gauze through which the citrated blood is to be filtered from the original flask into the syphon bottle.

(h) One nursing bottle into which the blood is filtered and from which the blood is introduced into the patient's circulation.

This bottle is to be fitted with a two hole rubber stopper into which is introduced 1 straight glass tube and 1 bent tube, the latter being connected



Fig. 19.—Bottle, rubber tubing and needle connected.

with rubber tubing 2 to 3 feet in length, to the end of which is attached a metal tip (Fig. 18). A clamp should be attached to the rubber tubing.

The metal tip must fit snugly into the needle by which the blood is introduced into the child. A special adapter may be used to connect the metal tip with the needle.

*Technic.*—A Wassermann test should be done on every donor before his blood is used. If the blood is to be introduced intramuscularly or subcutaneously, no blood grouping or matching is necessary nor is sodium citrate necessary to prevent the blood from coagulation. In that case, blood is removed from the donor's vein by venipuncture needle connected to a Luer syringe, a tourniquet being applied an inch or two above the site of the puncture. As soon as the syringe is filled with blood, it is detached from the needle, given to an assistant, who injects the blood into the child's buttocks

and meanwhile the operator fills the second syringe with blood. This is repeated until the desired amount of blood is given.

The donor is preferably seated near a table with the arm from which the blood is removed held firmly at the edge of the table.

Needless to say that every precaution is to be taken to keep the field of operation and the blood sterile.

If the blood is to be introduced directly into the child's circulatory system, the blood of the donor and the child should be grouped or matched before the transfusion is undertaken. (Technic of blood matching described in article 2). If the child is below one month of age, no grouping or matching is necessary.

The blood removed from the donor by venipuncture is introduced into the flask containing the sodium citrate. The blood is then filtered through gauze into the nursing bottle.

Another needle is now introduced into the vein or into the longitudinal sinus of the child. The clamp over the rubber tubing is loosened and the blood allowed to run to the tip in order to expel air bubbles. (Fig. 19.) The blood is now run in from the inverted nursing bottle into the child's vein or sinus by means of gravity. On completion the site of puncture is sealed by collodion.

*(To be continued.)*



# LABORATORY METHODS

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## A SIMPLE METHOD FOR COMPUTATION OF METABOLIC DETERMINATIONS BY THE USE OF THE SLIDE RULE\*

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By M. W. HOLLINGSWORTH, M.D., ANAHEIM, CALIF.

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THE long fraction needed in the computation of the basal metabolic rate has turned the attention of several writers toward some simplification of its solution. Recently, Newcomber arranged the necessary data in the form of several condensed tables and graphic curves. Some of these are constructed to read directly in logarithms, which facilitates the finding of these figures. However, any method requiring the use of the logarithms as such necessarily calls for the writing down of all the figures, adding them up, and the conversion of this sum which is also a logarithm, into the answer. J. H. Smith and M. C. Smith devised a very ingenious graph by which one arrives at the basal rate. This graph, however, makes arbitrary the use of one standard, but is accurate to within  $\pm 1$  per cent. McCaskey has simplified the calculation somewhat by using tables and a hypothetical constant. He has made optional the use of logarithms but the calculations in his formula are more quickly performed by using logarithms, so there is little, if any, actual saving of time or labor.

One year ago I instituted the use of the circular slide rule† in the laboratory of this clinic for all mathematical problems, including the determination of the basal metabolic rate. We are able to reduce the data concerned in the determination of the basal metabolic rate to the answer in less than one minute, without jotting down any intermediate data, or any logarithm whatever. Occasional checking up by logarithms has shown absolute agreement in the computations. The use of the slide rule does not limit one to any particular normal standard; one may use the twenty-four hour output of calories or the oxygen consumption per minute with the same degree of accuracy. The circular slide rule has the advantage over the straight one in being applicable to the solution of any fraction, no matter how long, without setting down any intermediate answer to use as a new starting point. One never runs off the scale of the circular instrument as it carries the problem along continuously to the end.

There are two types of the circular slide rule on the market. One bears close resemblance to the straight pattern in having two concentric discs

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†I realize I am taking some liberty with the English language in speaking of a circular rule. I do this because the circular instruments follow the same principle as the straight ones.

comparable to the two rules of the straight slide rule, which can be either rotated independently of each other, or in unison. There is also pivoted to the center a transparent sector carrying a hair line, which is the counterpart of the slide on the straight type, and which (like the slide on the straight rule, this transparent sector) can be moved independently of either disc. It also has the great advantage of having a convenient thumb nut by which the sector can be quickly clamped to the outer dial for making calculations requiring a common divisor or multiplier. The concentric discs are graduated in the same manner as the gradations on the rule and the co-ordinated rule of the straight variety. This circular instrument particularly simplifies problems for the beginner as the sector is lettered opposite the places to set the numbers, thus obviating any necessity of memorizing directions. The sector is lettered "N" opposite the place on the hair line under which the multiplicand or dividend is placed ("N" literally representing the number one is working on), and "M" and "D" opposite the places in each instance where the multiplier or divisor is placed. A conspicuous arrow, marked "Answer," points to the answer on the outer dial, while a smaller arrow on the outer dial marked "Proof," gives a check answer to be read on the inner dial. For practical purposes one neglects the proof answer entirely. This type of computer is practically foolproof. Fractions such as

$$\frac{2160 \times .923 \times 4.825 \times 1440}{10.03 \times 1000 \times 1270}$$

where one usually resorts to logarithms may be reduced by simply manipulating the sector and the discs alternately, the final answer being obtained without requiring the consideration of intermediate answers.

The other type of circular computer has only one disc, but it has two transparent arms or sectors. These are pivoted to the center, the one superimposed over the other like the hands of a clock. The construction of the pivot is such that the short arm may be rotated independently of the longer without disturbing the relation of the latter to the disc. However, rotation of the long arm automatically carries with it the short one, causing both to move as one. There is no lettering on this computer to assist one. The principle upon which this rule is based necessitates the rotation of one of the arms from the original setting in turning to the answer of all simple problems of division or multiplication. If there is any doubt one cannot simply look around the scale to verify the original setting as with the double disc, but must move the arms away from the answer back to the setting. For reckoning continuous fractions the single disc type is just as convenient as the other since we do not read our answers until the end.

There is nothing formidable about the use of the slide rule. The application of the circular type to medical problems saves so much time that I will illustrate the use of both instruments described above in the calculation of the basal metabolic rate from the findings of an instrument of the portable Benedict type; it works equally well with any other type of apparatus.

We usually compute the rate for the previous ten minute period as each consecutive period is being run, and determine the last as the patient is dressing.

We will suppose that in 10 minutes,  $1\frac{4}{5}$  seconds, the patient consumes 2160 mls of oxygen with the barometer standing at 754 mm. of mercury and with the temperature of the gasometer at  $20^{\circ}$  C. We will assume a theoretical rate of 1270 calories per 24 hours. Our problem is:

$$\frac{2160 \times .924 \times 4.825 \times 1440}{10' 1\frac{4}{5}'' \times 1000 \times 1270}$$

We reduce  $1\frac{4}{5}$  seconds to its decimal equivalent in minutes by placing the hair line of the sector over 18 ( $1\frac{4}{5} = 1.8$ ) and turning the inner disc until 60 is under the hair line opposite "D." The answer points to 3 which is the answer after placing the decimal, becoming .03 minutes (one places the decimal either by simple inspection or by following certain directions that accompany the instrument; very little practice enables one to correctly point off the decimal from inspection). This simplifies our fraction which now stands:

$$\frac{2160 \times .924 \times 4.825 \times 1440}{10.03 \times 10000 \times 1270}$$

In our actual work we have this problem memorized and set down only the barometer correction as such, the duration of the test which is reduced to decimals directly from the stop watch, and the theoretical calories which all become an integral part of the final report. Taking up the solution of this continuous fraction we place the hair line of the sector over 216 opposite "M." move the inner dial until 924 is under the hair line opposite "M." then hold the two dials together and move the sector till the hair line is over 1000 on the outer dial, then turn the inner dial until 144 is under the hair line opposite "N" of the sector. At this point, the 24 hour calories produced by the patient may be read on the outer scale where the arrow points. This is unnecessary however in figuring the result. I merely mention it as we usually write this data on the report. Next hold the two dials together and move the sector until the hair line is over 127 on the inner dial opposite "M." the answer is now read under the hair line on the outer dial which is in this case 1087 or 108.7 per cent, or, as is usually given, plus 8.7. The actual working out of the test consumes less than a minute which is less time than it would take to write down the logarithms.

We will now undertake the solution of the problem with the other rule. This rule has the two arms I spoke of, one being a little longer than the other; moving the longer arm carries with it the shorter, or the shorter may be moved alone. Both arms have hair lines. Taking first the duration  $10' 1\frac{4}{5}''$  place the hair line of the long arm (which we will designate as "L") over 60 and the hair line of the shorter arm (which we shall designate as "S") over 1.8 then move "L" until its hair line is over 10, this carries

with it "S" the hair line of which comes to rest over 3 which is the answer after pointing off the decimal.

Applying this rule to the solution of our long fraction, turn "L" until its hair line is over 216, turn "S" until its hairline is over 1003, then turn "L" until the hairline of "S" (turning "L" turns "S" with it as I mentioned above) is over 924, then move "S" until its hair line is over 10, turn "L" until the hair line of "S" is over 4825, then turn "S" until its hairline is over 127, now turn "L" until the hair line of "S" is over 144, and under the hair line of "L" we find the answer, 1088 which on being reduced to decimals (or deducting the 100 per cent) gives us our usual method of expressing the basal metabolic rate as plus 8.8.

We will now check up this problem by using logarithms. Taking the logarithms of the numerators we find them to be as follows:

$$\begin{array}{r}
 2160 - 3.33445 \\
 .924 - 1.96567 \\
 4.825 - 0.68350 \\
 1.440 - 3.15836 \\
 \hline
 \text{sum} \quad 7.14198
 \end{array}$$

Logarithms of the denominators:

$$\begin{array}{r}
 10.03 - 1.00130 \\
 1000 - 3. \\
 1720 - 3.10380 \\
 \hline
 \text{sum} - 7.10510
 \end{array}$$

$$\begin{array}{r}
 \text{Subtracting } 7.14198 \\
 7.10510 \\
 \hline
 \end{array}$$

.03688 equals logarithms of 1.086 or - 8.6

This confirms the answer we obtained by the use of the slide rule. It requires but a very little practice in the use of the rule to make it indispensable to anyone dealing with mathematical problems requiring division or multiplication. Almost anyone can learn to use it in 20 minutes. For working out graphs it saves hours of labor. The first rule mentioned comes with an attachment or clamp by which it can be fastened to the ledge of a desk and operated entirely by one hand.

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## NEW ANIMAL HOLDERS AND MOUTH GAGS\*

BY JOSEPH LEPAK, CHICAGO, ILL.

LABORATORY workers have constantly been devising simpler methods for their experimental work, and especially is this true with animal work. Heretofore it has required two or possibly more attendants to perform an intravenous injection in a guinea pig; or gastric feedings in dogs or rabbits by means of a stomach tube have been a trying, and, in fact, a troublesome procedure. During the course of recent experimental work in this laboratory it became evident that our instrumental equipment for these experiments was inefficient. It is therefore the purpose of this report to describe three



Fig. 1.—Diagram of mouth gag for dog. The horizontal bars are covered with rubber tubing.



Fig. 1A.—Mouth gag in position, showing upper and lower horizontal bars behind canine teeth. The most circular shaped bars are placed at the sides of the dog's face.

instruments which we have devised, and used with very satisfactory results for the past 18 months. These instruments are not only simple in their construction, but they also do away with the excess help that was previously required.

### CONSTRUCTION OF MOUTH GAG

This instrument is of two sizes; the larger being used for the dog (Fig. 1), the smaller for the rabbit (Fig. 2). The dog mouth gag is made of iron rod about  $\frac{1}{8}$  inch in diameter. It is curved to an almost semicircular shape to fit the sides of the dog's mouth (Fig. 1A), and as the rod is brought forward, the two front ends are attached to their corresponding sides in a horizontal manner, the left upper to the right upper and the left lower to the right lower. These horizontal bars are parallel with each other and cov-

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ered with rubber, so as to prevent any injury to the dog's teeth or lips. This instrument can be constructed by almost any laboratory worker, the size depending upon one's own individual judgment.

The rabbit's mouth gag is similar in a way to that of the dog's mouth gag, but instead of having the curved sides there are only the two parallel horizontal bars, connected by a V-shaped support (Fig. 2A). The rabbit's mouth gag is made of a heavy wire rod, about  $\frac{1}{16}$  inch in diameter.

These instruments constructed on a smaller scale can be used as mouth gags for guinea pigs, mice, or cats.

*The Use of the Mouth Gag.*—The animal, dog, or rabbit, is placed between the legs of the attendant, and putting the lower horizontal bar of the gag in the animal's mouth and forcing the lower jaw downward, the instrument slips just behind the canine teeth of the maxilla; the upper bar of the gag is slipped into the animal's mouth and the upper teeth are supported on the

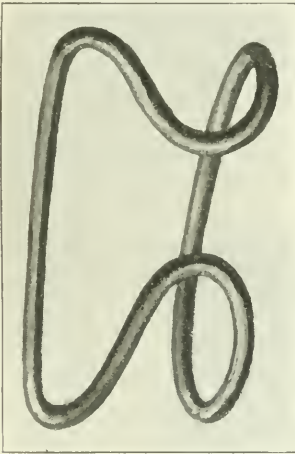


Fig. 2.—Diagram of rabbit's mouth gag.



Fig. 2A.—Mouth gag in position.

upper bar. In this way the animal's mouth is kept open without any injury to the soft tissues or teeth. The same attendant holding the dog can pass a stomach tube or perform any other work in the animal's mouth. This method is very simple and does away with the extra help that was previously required.

#### GUINEA PIG HOLDER

This instrument consists of an ordinary hemostat to which is attached (by means of soldering) a wire rod. These rods extend forward and cross each other in the form of an X (Fig. 3). A semicircular, solid, shaped piece of tin about  $1\frac{1}{2}$  inches wide and 2 inches long is soldered to the union of the hemostat and wire. The winged-shaped tin is attached perpendicular to the hemostat.

*The Use of the Guinea Pig Holder.*—The animal is tied down to the board in the usual manner. The guinea pig holder is put around the animal's head (Fig. 3A) and this brings the field to be operated upon forward. This method

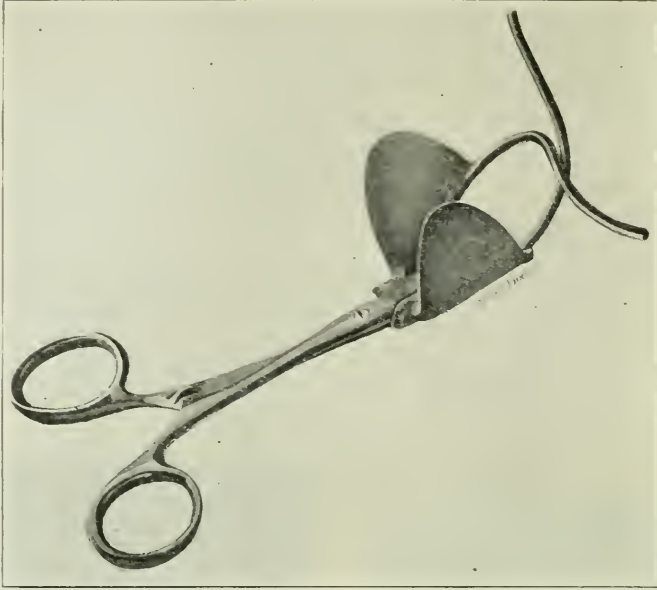


Fig. 3.—Diagram of guinea pig holder. The wires cross each other to form an X. The semi-circular tin shaped wings are attached to the junction of the hemostat and wires.



Fig. 3A.—Guinea pig holder in position showing the wing-shaped tin on each side of the guinea pig's head. The X shaped portion is behind the guinea pig's head, thus elevating the neck and bringing the field to be worked on forward. The hemostat is locked and there is no pressure on the animal's head or neck.

does away with an extra attendant holding the guinea pig's head, and also prevents the animal from biting any one working with it. We have used this holder particularly in the use of intravenous injections.

#### SUMMARY

Three instruments are described, one a dog and a rabbit mouth gag; the other a guinea pig holder.

These instruments are very simple to make and are great aids in saving time, simplifying the work, and decreasing the number of attendants that are ordinarily required in feeding animals by stomach tube, or intravenous injections in guinea pigs.

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### TECHNIC FOR THE REMOVAL AND TRANSPLANTATION OF RAT AND MOUSE TUMORS\*

BY DONALD C. A. BUTTS, PHILADELPHIA, PA.

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A TECHNIC which yields a high percentage of successful transplantations with comparative ease, and is sufficiently rapid to prevent probable loss of identity of tumor type is a factor of utmost importance. Especially is this necessary in the smaller laboratory, where the supply of tumor-bearing animals is limited and the necessity of successful inoculations is imperative.

In this laboratory we found it necessary to devise a means of tumor transplantation whereby the original animals bearing tumors might be maintained, and tumor inoculations from such animals yield a high percentage of unaltered successful "takes," in contrast to the method employed in the larger laboratories, which have an unlimited number of animals bearing the same strain of tumor at their disposal. In such cases the usual procedure is to decapitate the animal prior to removal, pathologic examination and transplantation of the tumor.

The technic employed in this laboratory is as follows:

The animal bearing the tumor (rat or mouse) is anesthetized with ether and tied, by the legs, to an animal board; the area over and directly surrounding the tumor is then carefully clipped free from hair, as shown in Fig. 1. The area is then washed with 95 per cent ethyl alcohol, painted with tincture of iodine and allowed to dry.

A smooth, rounded block is placed under the back of the animal, directly below the tumor, in order to elevate and steady the site of operation, thereby assuring greater ease to the operator.

The anesthesia, which is administered by placing the head of the animal into a cylindrical cardboard container, in which a large piece of cotton is saturated with ether, must be carefully regulated. As soon as complete general anes-

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\*From the Pennsylvania Department of Health Laboratories.  
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thetia is effected the cylinder is removed and replaced with an empty cylinder. If there is any indication of the animal returning to consciousness the cylinder containing the ether should be replaced; however, signs of recovery from the ether must not be confused with those demonstrated by the administration of too much anesthetic. Generally the former condition is demonstrated by signs of pain and squealing, while, in the latter, respiration is usually very rapid, shallow and difficult, associated with quick and repeated



Fig. 1.

convulsive movements. If this latter condition persists after discontinuance of the ether, a small quantity (0.5 c.c.) of sterile saline solution may be injected, usually subcutaneously. Therefore proper anesthesia, with special care during the induction, is essential.

Before starting the operation all instruments and materials to be used should be sterilized in the regular manner. This is imperative, otherwise severe postoperative infections may result.

The following constitutes the necessary material to have on hand when starting the operation: 1 small scalpel; 2 dissecting forceps; 3 hemostatic forceps; 1 pair iris scissors; 1 tube of No. 4 silk; 1 tube of No. 00 catgut; 1 curved suturing needle; 1 sterile Petri dish; 1 lumbar puncture needle; 1 Luer syringe (1 c.c. capacity); flask of sterile saline solution; sterile cotton; bottle of 95 per cent ethyl alcohol; small bottle of formalin solution (4 per cent).

To perform the operation, the operator and assistant take their positions on opposite sides of the table or animal board. The incision is made directly over or at the base of the tumor. When the tumor is large with the central portion necrotic, an incision around one-half the diameter at the base of the tumor is advised; under other conditions an incision directly over the tumor is usually employed; however this point remains one of choice to the operator.

Each edge of the incision is grasped with a hemostat and carefully held by the assistant. The skin is incised until the encapsulating membrane of the tumor is reached (Fig. 1A). The assistant now grasps the tumor and draws it up and towards him, while the operator draws the border of the incision nearest to him down and away from the tumor. In this manner the subcutaneous tissue may be separated from the tumor on one side and by reversing this manipulation the other side of the tumor is freed from its subcutaneous connection (Fig. 1B). The tumor is now held between the thumb and index finger and the remaining connections severed with scissors or scalpel (Fig. 1C). When completely excised it is placed into a sterile Petri dish.

Slight bleeding encountered during the operation is controlled by immediate compression, whereas, profuse bleeding necessitates ligation of the bleeding points with silk thread. After all bleeding has been checked and the excess skin is clipped off (indicated by dotted line, Fig. 1C), the wound is closed by suturing the borders of the incision with No. 4 silk or No. 00 chromic catgut (interrupted sutures are employed), the number depending upon the size of the incision, one stitch being required for each  $\frac{1}{4}$  inch (Fig. 1D). The line of incision is painted with tincture of iodine and the animal placed in a separate cage until entirely recovered from the anesthetic, usually about twenty minutes. No dressings are necessary following operation; food and water are permitted. The time occupied in performing the operation, including etherization and suturing, is generally about fifteen minutes.

The edges of the incision in cases where the tumor is not adherent to the skin, are healthy and heal by first intention; but in cases where the skin has become adherent and more or less necrotic, sufficient cutaneous tissue must be removed with the tumor in order to secure smooth, regular and healthy edges to permit suturing.

*Inoculation of the Tumor.*—The method of inoculating the animals is the one generally employed and will be outlined as follows:

The tumor is held with sterile forceps and cut through the center horizontally to the long axis with a sterile knife or scalpel. One-half is placed in the formalin solution for sectioning and pathologic examination, while the other half is used for the inoculations. A portion of the border is cut free

from necrotic matter which may be present, and cut into small pieces (about 0.03 gm., or about one-half the size of a pinhead). A lumbar puncture needle is flamed in the Bunsen flame and wiped off with a piece of cotton soaked with saline solution and then with a piece of cotton moistened with ethyl alcohol. The fragment of tumor is introduced into the bore of the needle with the point of the scalpel. The point of the needle is introduced subcutaneously at the groin and pushed up to the axilla where the piece of tumor is deposited by pushing in the plunger of the needle. If the transplantation is successful, a nodule can usually be found in the inoculated animals after fourteen days.

After using this method of excision and inoculation over a period of two years, in which time over two hundred tumors were removed and approximately one thousand animals inoculated, there were only two deaths as the result of operation, both due to poorly regulated anesthesia. In no case have the inoculated animals developed any infection and have yielded an extremely high percentage of successful transplantations.

Other advantages possessed by this technic are, that a small fragment of the tumor, or where multiple tumors exist, several may be left in the host unaltered for further observation and transplantations. Moreover, the normal increase in this colony is not inhibited, evidenced in the animal represented in Fig. 1, which gave birth to six young directly after operation, the nursing and care of which were normal in every respect.

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## EDITORIALS

### *Vaccine Treatment in the Presence of Infection*

IN a recent series of articles Wright has ably summarized his investigations and conceptions with regard to therapeutic inoculation. Although Jenner stands as the originator of vaccination, it was Pasteur who first developed a clear understanding of the principles involved and applied them, particularly in his work on anthrax and rabies. Pasteur's conceptions may be briefly summarized somewhat as follows. In prophylactic inoculation, the pathogenic microorganism, or in case such is unknown, the virus containing the specific pathogen must be obtained. The vaccine must consist of the living germ whose virulence has been attenuated. The exact amount introduced into the animal to be immunized is not of great importance. Vaccines should be given only to uninfected animals, or if infected, only in case the incubation period still has more than ten days to run. It should be administered subcutaneously. Protection is obtained only after ten or more days from inoculation and is specific in that vaccination with one pathogen will not protect against another unrelated organism.

Following the work of Pasteur and particularly in the development of typhoid immunization, it developed that the antigen need not necessarily be



a living attenuated microorganism, but may consist of killed virus. In fact, it was soon recognized that dead virus was more active in producing immunity. The explanation appears to lie in the principle formulated by Ehrlich; *corpora non agunt nisi soluta*.

In typhoid vaccination it was found that better results were obtained when a severe reaction was avoided. The bactericidal power of the blood passes through a negative phase following administration of quantities of typhoid vaccine great enough to produce severe constitutional reaction. Following this there comes a positive phase in which the bactericidal power may be increased as much as a thousand times. With large doses the negative phase becomes protracted and may sometimes last indefinitely. Following doses which produce a minimum of constitutional disturbance, the negative phase disappears, the positive phase beginning immediately, so that often as soon as twenty-four hours after inoculation, the bactericidal power of the blood is decidedly enhanced. This fact necessitates some change in the Pasteurian code, since vaccine therapy may, theoretically at least, be given with good results when the incubation period has much less than ten days to run. This has occasionally been demonstrated to be true in inoculation during the incubation period of the plague. Presumably the immunizing process occurs in those areas of the body which are still uninfected. Furthermore, the quantum of vaccine administered, contrary to the tenets of Pasteur, is of considerable importance in determining the rapidity of rise in bactericidal power and the presence or absence of a negative phase.

Wright's early modification of the Pasteurian code contained the following essential points. We must possess the microbe or the virus of a particular disease in order to manufacture a vaccine. Stock vaccines made from the specific microorganism give results comparable to those obtained from autogenous vaccines. The vaccine should, wherever possible, be sterile. Vaccines may be used in prophylaxis, in preventive treatment during the incubation period of general infections and in localized infections not accompanied by fever and frequent autoinoculations. Bacterial vaccines should be incorporated hypodermically. Small doses of vaccine produce a positive immunizing effect within twenty-four hours. Larger inoculations, like heavy autoinoculations, result in a negative phase which is more pronounced the greater the quantity of antigen.

The dosage should be that which promotes the optimum immunizing or "epiphyllaetic" response. If in obtaining this optimum result, sufficiently large doses are necessary to produce a temporary negative phase, the latter is permissible in view of the end result desired. Reduced doses should be employed when the chief matter of concern is to obtain rapid clinical improvement. When the patient is infected, the dose should stand in inverse relation to the volume of his infection.

Wright also hypothecated in his earlier work that the anti-bacterial substances elaborated in response to inoculation operate specifically upon the variety of microbe which has furnished the vaccine with the possibility of some slight collateral immunization.

While this second code of Wright's differs quite radically from the Pasteurian code, his third is at even greater variance with the second. First he assumes that although the nature of the infecting microbe should be known, it is not necessary that the vaccine be derived from the species causing the disease. It is sufficient to have demonstrated that the vaccine used, no matter what its source, increases the concentration and action of those anti-bacterial substances which operate upon the specific infecting microbe. This hypothesis tends somewhat in the direction of the more recent conjectures with regard to so-called nonspecific immunity.

The second radical departure lies in the method of inoculation. On the assumption that the bactericidal substance is produced in the blood, direct introduction of the vaccine into the blood stream will call forth prompter reaction. "When vaccines in appropriate doses are added to the blood either *in vivo* or *in vitro*, instantaneous epiphyllactic or immune response is evoked and the maximal response may be expected after only very short delay."

This reaction, according to Wright, consists in an extrusion of bactericidal elements from the leucocytes. To only an insignificant extent is the germicidal action due to phagocytosis. The anti-bacterial substances derived from the leucocytes are polytropic, exerting their action not only upon homologous but also upon quite unrelated species of microbes. Wright finds that normal blood is much more highly destructive to staphylococci than is blood from a case of streptococcus septicemia. In the latter blood there is an impairment of the destructive mechanism against microorganisms other than that causing the infection.

In septicemias and other heavy bacterial infections, the leucocytes lose their power of responding to vaccines. Here it is necessary to determine whether the blood still retains its power of epiphyllactic or immune response. If it does, vaccination may logically be employed even in these conditions. Where there is no such response the appropriate treatment would be by immuno-transfusion, transfusion with healthy blood which has made the proper epiphyllactic response.

Wright finds that in normal bloods, two bactericidal mechanisms play a part. If two specimens of the same blood be implanted with bacteria, the first with a few, the second with a large number, one would expect to find a greater proportion of bacteria destroyed in the former. As a matter of fact, the germicidal efficiency of the serum is greater with the higher implantation. The presence of large numbers of bacteria apparently calls forth some secondary immunity reaction which remains inactive when only small numbers of bacteria are present. With implantations of 1000 staphylococci per c.c. an average killing of over 75 per cent is observed, while with implantation of less than 150 staphylococci per c.c., none were killed. The suggested interpretation is that we have in the blood an apparatus for primary and one for secondary defense, termed aphylactic and epiphyllactic. The former probably depends upon phagocytic action and the bactericidal action of serum and deals with small bacterial implantations. The epiphyllactic response develops from anti-bacterial elements given out by the leucocytes when these are incited by

antigen. The epiphylactic response is far more efficient than the aphyllactic. Blood implanted with 33 microbes per c.c. cannot kill one, but when implanted with 10,000, it can kill 2,000. This indicates that some new method of response has been evoked.

In streptococcus endocarditis where there are few microorganisms in the blood, with only moderate pyrexia, little constitutional disturbance and a long standing progressive course, the machinery of first defense has failed to perform its work and the epiphylactic machinery has never been called into action.

In those cases of bronchopneumonia which recover, Wright hypothesizes that the epiphylactic machinery has been effectively although tardily brought into operation. In the fatal cases, this higher response has been put out of action by overwhelming bacterial intoxication. In septic cases, such as advanced tuberculosis and localized abscesses, the epiphylactic defense reaction is called forth whenever large amounts of bacteria are delivered into the blood stream and with excellent though temporary results.

The question naturally arises as to what is the proper vaccinating dose in a given case of infection. The proper dose would be the number of bacteria which would, together with the bacterial elements already in circulation, evoke in the patient's blood a maximum immunity response. In prophylactic inoculation, the active principle supplied from without will not be supplemented from within and we may therefore employ a considerable dose of vaccine. In infection, the volume thereof must be considered and a proportionately smaller amount of vaccine administered. When a hyper-vaccinating dose is already circulating in the blood, we should abstain from therapeutic inoculation.

The dead microbes provide the therapeutic agent but the living decide the issue. The magnitude of the response reaction will depend upon the dose of vaccine, but the effectiveness thereof depends upon the number of living microorganisms to be dealt with. There is an upper limit to the number of bacteria in the blood which can be taken care of even by the mechanism of the epiphylactic response. Laboratory experiments would indicate that the epiphylactic reaction can control infection with hundreds or perhaps thousands of bacteria per c.c., but it is not competent to control heavier infections. However, even in the severe streptococcus septicemias, the blood infection rarely exceeds several hundred per c.c. But it is more difficult to attack the millions of microorganisms not in the blood stream, but growing in body tissues.

Wright's work indicates that the killing of the bacteria in the epiphylactic reaction is practically instantaneous, fully two-thirds being destroyed in this way. That the germs are killed instantly and at ordinary temperature gives conclusive evidence that phagocytosis is not the cause of their destruction. This latter phenomenon begins only after an appreciable delay, especially at room temperature. The conclusion reached is that the action depends upon some bactericidal property of the serum. This high bactericidal power is not present normally but suddenly develops upon the implantation of large



concentrations of bacteria. The body tissues play no part in the sudden access of power, because it can occur *in vitro*. Wright believes that this increased bacterial efficiency depends upon the extrusion from the leucocytes of a nonspecific destructive poison. In support of this he draws attention to the fact that when washed leucocytes are implanted on cultures of staphylococci or streptococci, the latter are killed. This destruction occurs without phagocytosis.

This opens the way to an understanding of the possible existence of non-specific immune reactions. The hypothetical substance which kills in the epiphylaetic response will act upon all foreign bacteria. The anti-bacterial agent is polytropic. Again in favor of this is the observation that the blood kills instantly, before time has elapsed for the elaboration of a specific substance. We can more readily conceive of a general immune substance than of a specific reagent for each and every antigen. This at least, as far as elements to be derived from the leucocytes are concerned. Thus it has repeatedly been observed that an individual does not develop more than one exanthem at a time.

The suggestion is made that the leucocytes may be stimulated to the extrusion of the bactericidal substance by chemical agencies. Salvarsan in syphilis complicated with streptococcus infection may eliminate the latter. Blood for a few hours after the administration of salvarsan becomes bactericidal to streptococcus and staphylococcus. The elaboration of diphtheria antitoxin and of agglutinins to the colon bacillus may be appreciably increased by the exhibition of manganese chlorid. The editor observed in one of the camp hospitals during the influenza epidemic that those patients who had recently received salvarsan were less prone to ward infection with influenza than were those who had not been so treated.

Large doses of vaccine administered subcutaneously are fed slowly into the blood stream, and if the rapidity of absorption into the blood happens to be just right, as good results may be obtained as by the intravenous injection of the proper dose for epiphylaetic response. On the other hand, if the vaccine enters the blood too rapidly a formidable negative phase may supervene. Finally, if a relatively small dose is given subcutaneously, the concentration in the blood may never reach that desired for maximum response.

Wright has developed a "vaccine response test" by which he determines *in vitro* whether a patient is or is not capable of making an immunizing response. When such response can be made, it is possible in addition to ascertain the requisite dose of vaccine which, when injected intravenously, will produce the optimum reaction. In those cases in which the response action will not occur, the transfusion of immune blood may be immediately resorted to without wasting more time on vaccine therapy.

In this procedure the first fact to be ascertained is the bactericidal efficiency of the patient's blood. This is done by comparing with the efficiency of a control blood. A micromethod is described, utilizing 50 c.mm. of blood. This test requires twenty-four hours' incubation, and in urgent cases may



be dispensed with. The second step in the determination of the immune response reaction consists of a series of laboratory tests termed the "chiastic method." Four phagocytic mixtures are made, the first containing patient's serum and washed leucocytes, the second containing normal serum and washed leucocytes, the third patient's serum and normal leucocytes, and the fourth, normal serum and the patient's corpuscles. The mixtures are made in equal proportions and to each is added a similar volume of a suitable bacterial mixture. These are incubated and read as in the familiar technic for the determination of the opsonic index. The *opsonic index* of the patient's serum is obtained by dividing the phagocytic count of preparation 3 by that of preparation 2; the *phagocytic index* of the patient's blood, by dividing the phagocytic count of preparation 1 by that of preparation 2. The *phagocytic efficiency* of the patient's leucocytes is expressed by the fraction obtained by dividing the count of preparation 4 by that of preparation 2. According to Wright, the premonitory signs of a grave infection would seem to be a reduction of the leucocytic efficiency coupled with a rise in the opsonic index and a moderate fall in the phagocytic index. In the later, graver stages of infection, the opsonic power is also reduced and the whole triad of phagocytic function gradually falls until at the extreme limit, zero is reached in each case.

In those cases where the bactericidal power of the blood has been found deficient, it remains to determine whether the patient has any capacity of making immunizing response and with what vaccine and what dose he can make the optimum response. This is done by the introduction of graduated amounts of vaccine into a series of volumes of the patient's blood and seeing whether there is any improvement in the hemobactericidal or phagocytic power of the vaccinated blood or in the serobactericidal or opsonic power of the serum. If time does not permit the culturing of the blood for determination of bactericidal power, we may utilize the measurement of the phagocytic power of blood and the opsonic power of serum to which the graduated doses of vaccine have been administered. Thus the phagocytic index of one patient's unvaccinated blood was 1.8. The same blood inseminated with twenty, forty and eighty streptococci per c.c. showed a phagocytic index of 1.6, 1.9 and 1.4 respectively. This showed no advantage over the uninoculated blood. However, the same blood vaccinated with 160 per c.c. showed an index of 3.2, a decidedly better response reaction. The conclusion was that the patient's blood still possessed a considerable capacity for immunizing response and that vaccination with 160 killed streptococci per c.c. would give the best response. The patient was therefore inoculated intravenously with 700,000 streptococci with excellent clinical results.

The three sequential points enumerated by the author in the treatment of infection are, first, the removal of localized accumulations of pus; second, intravenous administration of vaccine in those cases which are found capable of immunizing response; and third, immuno-transfusion for those cases so overwhelmed with infection that they are incapable of producing an optimal epiphyllactic response reaction.

The author reports cases showing improvement in the phagocytic power or index of the patient's cells after evacuation of pus, after therapeutic inoculation and after immune transfusion. The donor for transfusion is treated by intravenous inoculation of that dosage which has been found to call forth a maximum epiphylactic reaction in his own blood. He is bled one-half hour later.

Whether or not Wright's work of the past few years, as summarized in his series of articles, becomes generally accepted, it does open several interesting avenues of thought toward which many immunologists are independently turning. These may be summarized as follows.

The results of vaccination in the presence of infection are not always due to a specific antigen-antibody reaction.

The quantum of vaccine to be administered is all important and must be determined for each case. It may be incorporated intravenously.

This quantum may be roughly determined within a short period by the measurement of a series of phagocytic reactions.

#### REFERENCES

- Wright, Sir Almroth E.: New Principles in Therapeutic Inoculation, *Lancet*, London, Feb. 24, 1923, cciv, 365.  
 Wright, Sir Almroth E.: *Ibid.*, March 3, 1923.  
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—W. T. V.

### *The Black Death of the Fourteenth Century*

OCCASIONALLY a physician meets with a layman who has read Hallam, Creighton, Gasquet, or some one else who has written on the epidemics of the middle ages, and the physician is asked, what was the nature of these diseases, are such still in existence, can they be identified, how do the symptoms as reported at that time correspond with those in evidence now, and do the older descriptions contain marked exaggerations? One of the great epidemics described by the authors referred to is known as the black death of the fourteenth century. We are told that this epidemic within a few years killed 25,000,000 people in Europe, reducing the total population about one-half. The literature concerning the black death is fairly satisfactory and enables us to trace it from Bagdad, or at least from that region to Europe, and to follow it as it flowed out to the north and west. At that time caravans carried the merchandise, which had been assembled at Bagdad, from China and India, by several routes to Europe. There were, however, two of these trails which were most generally taken. One followed the Tigris from the plains of Mesopotamia northward, passed over the mountains and reached the shores of the Black Sea at or about the present city of Trebizond. The other, better known and by far the shorter route, passed westward from Mesopotamia, finally reaching the eastern end of the Mediterranean Sea. Some of the merchandise passed around through northern Egypt from whence

it was shipped across the Mediterranean. For some years the epidemic had raged in India and China and, even as now, the virus had its home, or at least a home, in the Himalayas. At that time the rival cities of Genoa and Venice were in earnest contest, sometimes with military accompaniments, for the trade of the east. Merchant ships, often armed, were making regular trips across the eastern end of the Mediterranean. In the closing months of the year 1347 infected ships reached both Genoa and Venice. Gabriele de Mussi has left a graphic description of the epidemic as it entered Genoa. He tells the story as though he was on an infected ship. He writes: "Alas! our ships enter the port, but of a thousand sailors hardly ten are spared. We reach our homes: our kindred and our neighbors come from all parts to visit us. Woe to us, for we cast at them the darts of death! Whilst we spoke to them, whilst they embraced us and kissed us, we scattered the poison from our lips. Going back to their homes, they in turn soon infected their whole families, who in three days succumbed, and were buried in one common grave. Priests and doctors visiting the sick returned from their duties ill, and soon were numbered with the dead. O, death! cruel, bitter, impious death! which thus breaks the bonds of affection and divides father and mother, brother and sister, son and wife. Lamenting our misery, we feared to fly, yet we dared not remain."

In the *Decameron*, Boccaccio gives even a more striking picture of the plague as it occurred in Florence. The most frequently quoted paragraph from this racy author, so far as he treats of the black death, runs as follows: "What magnificent dwellings, what stately palaces, were then rendered desolate, even to the last inhabitant! How many noble families became extinct! What riches, what vast possessions were left with no known heir to inherit them! What numbers of both sexes, in the prime and vigor of youth, who in the morning, Galen, Hippocrates, or Esculapius himself, would have declared in perfect health, after dining heartily with their friends here, have supped with their departed friends in another world."

Living amidst the plague in Parma, Petrarch wrote: "Alas! my beloved brother, what shall I say? How shall I begin? Whither shall I turn? On all sides is sorrow; everywhere is fear. I would, my brother, that I had never been born, or at least, had died before these times. How will posterity believe that there has been a time when without the lightnings of heaven or the fires of earth, without wars or other visible slaughter, not this or that part of the earth, but well-nigh the whole globe, has remained without inhabitants?"

Probably there is not in the whole range of literature a more striking paragraph than the following written by an Irish monk: "And I, Brother John Clyn, of the Order of Minorites and the convent of Kilkenny have written these noteworthy things, which have happened in my time and which I have learned as worthy of belief. And lest notable acts should perish with time, and pass out of the memory of future generations, seeing these many ills, and that the world is placed in the midst of evils, I, as if amongst the dead, waiting till death do come, have put into writing truthfully what I have heard



and verified. And that the writing may not perish with the scribe, and the work fail with the laborer, I add parchment to continue it, if by chance any one may be left in the future and any child of Adam may escape this pestilence and continue the work thus commenced."

Other striking illustrations might be taken from the literature of the time, but we turn to inquire as to the nature of the black death. This inquiry is easily and certainly answered. Fortunately, Boccaccio was not the only chronicler of the epidemic as it ravaged Florence. There was in that city and at that time a more scientific observer. His name was Villani. This author says that the disease manifested itself in two forms. In one the lungs were evidently involved; there was coughing and spitting of blood, and from this form none recovered. In the other form, the glands in the inguinal and axillary regions were swollen, often ruptured and discharged, and from this form of the disease many recovered. At Avignon, then the seat of the papacy, there was the greatest surgeon of the time, Guy de Chauliac, physician to the pope and a man whose writings dominated surgical theory and practice for several centuries later. His testimony as to the nature of the disease is confirmatory of that furnished by Villani. Chauliac says that some patients had constant fever, accompanied by spitting of blood, and from this form of the disease all patients died in three days. Many, however, recovered from the bubonic form. Other Italian physicians repeat these descriptions so closely that there is no break in the character of the testimony. In France, Covino named the disease *pestis inguinaria*. In the remainder of Europe there appears to have been at that time no physician who recorded anything of value pertaining to the nature of the disease. The black death was without question the disease which we now recognize as the plague and it manifested itself, then as now, in both the pneumonic and bubonic form. It is worthy of remark that in the nearly 600 years which have elapsed since the prevalence of the epidemic about which we are now writing there has been no important or even recognizable change in the symptoms or lesions induced by this infection.

As has been stated, the black death found its way into Italy simultaneously by Venice and Genoa in the last month of the year 1347 or in the first month of the following year. We are somewhat surprised that it advanced so rapidly, reaching France and, indeed, spreading all over that country by the early summer and appearing at English seaports only a few weeks later. It spared neither city nor hamlet and it is interesting to make some inquiry concerning the living conditions of the people in Europe at that time. Recently, a Frenchman, M. Luce, has investigated this matter. He thinks that France at that time had a population of four or five million, possibly more. He says that there were numerous villages scattered rather thickly over the country, the most of which have long since disappeared. The huts of which these villages were composed were built of mud and clay, sometimes containing cavities filled with straw. As a rule, dwellings were of only one story. The public houses frequently had two. The roofs were thatched or covered with wood or stone. Windows were the exception and where



they did exist were mere slits in the clay walls closed with shutters. The doors were fastened with wooden latches, with open spaces above for the escape of the smoke, as in many of these huts there were no chimneys. It is more than likely, although on this point we have no direct testimony, that these huts housed more rats than human beings. We are told that before the black death appeared among the people in Florence large numbers of rats came into the streets in open daylight without apparent fear of man and that many of them died in the gutters.

Many cities or large villages in western Europe were so badly crippled by the black death that they have never fully recovered their position. Two years before this epidemic Yarmouth must have been a very flourishing English port: at least it contributed 43 ships and 1,950 sailors to Edward III who, at that time was moving on France, while at the same time London was able to supply only 25 ships and 662 mariners. Before the plague the great church at Yarmouth was not ample enough to accommodate the population. Since that time it has never been crowded. Before the black death it is said that the University of Oxford was attended by 30,000 students annually, a number never since approximated; indeed, in the year 1349 the king addressed the bishops and among other things he urged them to support the University of Oxford, which he stated was standing "like a worthless fig tree without fruit"

Do our older authors exaggerate the mortality caused by the black death? This is a question not easily answered. There were no vital statistics anywhere at that time. We can only guess at the population of England, France or Italy in the fourteenth century. Gasquet, a learned authority in church matters, has tried to figure out the mortality caused by the black death in England in this great epidemic. He concludes from a study of the monasteries and convents of that time that about half the population of England and Wales was destroyed by the black death. He thinks that before the plague the total population of England and Wales was somewhere between four and five million and he states that from the subsidy roll of 1377—some 27 years after the great mortality—the population was found to be 2,350,000. It is quite likely that the mortality among the religious orders was higher than it was among the people in general because of the crowded condition of the convents and monasteries. In England at least, the black death so thinned out the working classes that those who were left alive dictated their own wages and markedly improved their living condition. Gasquet says that in England alone about 25,000 priests were killed and that this so reduced the number of teachers in the classical languages that for the first time the vernacular was used in English schools.

—V. C. V.

## *The Standardization of Biological Stains*

ON March 2nd at the Chemists Club in New York City there was held a meeting of the Executive Committee of the Commission on Standardization of Biological Stains. The members of this Committee are: H. J. Conn, Geneva, F. B. Mallory, Boston, L. W. Sharp, Ithaca, N. Y., J. A. Ambler, Washington, D. C., and S. I. Kornhauser, Louisville, Ky. The meeting was also attended by C. H. Herty to represent the Synthetic Organic Chemical Manufacturers Association, and by F. P. Garvan and W. F. Keohan to represent the Chemical Foundation. The meeting is a matter of interest to everyone in the medical profession.

All physicians realize the need of dyes for staining specimens in the laboratory diagnosis and investigation of disease. It is not perhaps so generally realized that the dyes used for this purpose, in order to give constant results, must be of very precise chemical composition; and yet it is a very difficult matter for either the chemist or the biologist to control their composition. Before the war all stains were imported from a single German firm. This firm did not manufacture stains, but bought textile dyes in batches of considerable size, and after some preliminary testing bottled them and sold them under its own name to the biological laboratories of the world.

When the war broke out the American laboratory was deprived of this foreign source of stains. After the pre-war stocks already on hand had given out, much difficulty was experienced in getting stains of the quality necessary. The Society of American Bacteriologists began an investigation of American-made dyes that were being sold as biological stains. The results of this investigation were so promising that it proved possible to secure the assistance of the National Research Council through whose agency a cooperative investigation was arranged among the members of several national societies. Recently the work has been organized under a special commission independent of the Research Council but still representing the different national societies that were cooperating in the earlier work.

At the executive committee meeting of this Commission just held, the very encouraging results of the work were reported. It was shown that already the stains available in America are in practically all cases as good and sometimes better than the best of the pre-war stains. The most important fact brought out at this meeting was that while the pre-war stains were standardized only in an empirical way, by buying large batches without knowing the exact composition of the dye, they must now be standardized on the basis of pure chemicals.

The reason for this is because it is proving that in some cases the impurities present in the pre-war stains were very necessary. Sometimes these impurities were other dyes and sometimes supposedly inert materials like dextrin. In all such cases the task plainly before the Commission is to find out what the impurity is which was responsible for the good staining qualities

of the impure product. Then in the future the users of stains must demand that these impurities be present, not as impurities, but as intentionally added ingredients. When this has been done and the products are labeled and used accordingly, the American stains will become standardized in a true sense of the term.

Very shortly the Commission will begin issuing certification of definite batches of stain that it has found satisfactory. These stains will be put on the market under a special label bearing the name of the Commission. Users of stains must be on the lookout for products bearing this label. Buyers of stains must also watch for spurious imitations of this label put out by unreliable concerns. Any form of certification appearing on a stain label not bearing the name of the Commission is merely a certification by the manufacturer or dealer himself, and as such has no value.

The Chemical Foundation has very kindly agreed to support the work of the Commission financially.

—*H. J. Conn, Chairman, Executive Commission.*

## BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

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### *Clinical Medicine\**

IN this volume, the author comes into closer contact with his readers through the easy style of classroom conversation. It is true that in undertaking this method of presentation, one cannot go into exhaustive detail in the discussion of each subject. A critic might therefore complain of superficiality, but on the other hand, the important features and the newest facts are driven home in a manner that cannot be easily accomplished in more formal rhetoric.

In connection with the first case reported, the author applies his theories of the rationale of a diagnostic survey. He has discussed this subject often in the literature of the past few years, but in no place is it as concise and readily followed as in this volume.

The cases selected cover the various types of disease, so that the book as a whole, with its discussion of differential diagnosis, etc., forms an excellent teammate for a general treatise on internal medicine.

It is the type of book that one may pick up of an evening and read for pleasure.

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### *Thurston's Pharmaceutical and Food Analysis†*

ASIDE from the fact that the title of this book may be a little misleading, it is an extremely valuable volume. The first portion of the book deals with general methods of analysis and discusses in detail the polariscope, refractometers, specific gravity apparatus and other methods of analysis of fats, oils and waxes, such as the determination of acid value, boiling point, iodine value, saponification, etc. Following this is a chapter devoted to oils, fats and waxes in which the source, principal components, preparation, properties, uses and byproducts of each is thoroughly discussed. There follow chapters on dairy products, methods of analysis and detection of adulteration, and chapters on fleshy foods and eggs. The remainder of the book is taken up with a similar exhaustive study of the volatile or essential oils.

Although the book is not as inclusive as would be suggested by the title, it should be a valuable reference volume for workers in food and drug analysis. It had been the intention of the late author to cover the remaining aspects in a second volume.

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\*Clinical Medicine. Tuesday Clinics at the Johns Hopkins Hospital. By Lewellyn F. Barker, M.D., LL.D., Professor of Medicine, Emeritus, Johns Hopkins University; Visiting Physician to Johns Hopkins Hospital, Baltimore. Cloth. Price \$7.00. Pp. 617. Illustrated. Philadelphia and London, W. B. Saunders Company, 1922.

†Pharmaceutical and Food Analysis. A Manual of Standard Methods for the Analysis of Oils, Fats, Waxes, and Substances in Which They Exist; Together With Allied Products. By Azor Thurston, late chemist to the Ohio State Pure Food and Drug Commission. Cloth. Pp. 416. D. Van Nostrand Co., N. Y., 1922.



### *Laboratory Technique\**

THIS laboratory manual is the outgrowth of the systemization of the laboratory work at St. Luke's Hospital, New York. It covers the general subjects, histology, clinical pathology, clinical analysis, bacteriology and serology as applied in the routine work of a large hospital. In the general methods, there is little new, but the short cuts and "tricks" in examination so frequently worked out in individual laboratories and so seldom considered of sufficient importance to be put into print, are incorporated and make the book highly valuable for use in routine hospital work. The authors have realized that the index will be referred to very frequently and they have therefore placed it at the beginning of the book where it supplants a table of contents. This facilitates rapid reference. The book should be of particular value to the intern and to recently graduated physicians. An occasional typographical or composing error occurs, as at the top of page 24, but this does not detract from the value of the work.

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### Erratum

The third paragraph on page 308 of the article by J. D. Pilcher and Torald Sollmann, February issue, should read: A control test should always be made with silver nitrate, 0.15 mg. and 0.25 mg. per 10 c.c. If 0.15 mg. should give no gas; or should 0.25 mg. give more than a trace of gas, the inhibitory dose of silver nitrate for that yeast must then be determined, and the reading for the compound corrected by the formula: True inhibiting dose of compound : determined inhibiting dose of compound :: 25 : determined inhibiting dose of silver nitrate. For example a certain sample of yeast was inhibited by 250 mg. of silvol and by 0.40 of silver nitrate.

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\*Laboratory Technique. The Methods Employed at St. Luke's Hospital, New York. By F. C. Wood, Karl M. Vogel and L. W. Famulener. Second edition. Paper. Price \$1.35. Pp. 281. James T. Dougherty, New York. 1922.

# *The Journal of Laboratory and Clinical Medicine*

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## *ORIGINAL ARTICLES*

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### THE IMPORTANCE OF THE ADRENAL GLANDS IN THE ACTION OF CERTAIN ALKALOIDS\*

#### II. STRYCHNINE ON THE BLOOD PICTURE

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BY CHARLES W. EDMUNDS, M.D., AND PUTNAM C. LLOYD, A.B., ANN ARBOR,  
MICH.

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IN a recent paper by one of us (Edmunds<sup>7</sup>), it was shown that the adrenal glands were responsible for some of the effects which are produced in the body by the administration of certain alkaloids, such as pilocarpine, physostigmine and strychnine, which stimulate these glands to increased activity. All of these alkaloids have been shown by Stewart and Rogoff<sup>1, 2, 3</sup> to increase the amount of epinephrine in the blood; the first to a slight degree, the other two to a much greater extent. It became therefore a matter of much interest to see whether it would be possible to demonstrate a still wider effect of the epinephrine which is secreted under their influence and which might possibly explain some of the results which follow their administration.

One of the characteristic results of the administration of small quantities of epinephrine is the change which it produces in the total number of white blood cells and in the relative proportions of the different varieties. These changes have been described by several writers and are the subject of an extended study by Edmunds and Stone.<sup>4</sup> Briefly stated, in dogs the effect upon the blood of small doses of epinephrine is as follows: immediately following the injection the total number of leucocytes is increased, both lympho-

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\*From the Pharmacology Laboratory of the University of Michigan.

Received for publication, February 3, 1923.

The companion paper upon the effect of physostigmine upon the blood picture will appear in a subsequent number of this Journal.

cytes and polynuclear cells being involved in the increase. After this so-called first stage, the lymphocytes decrease in number while the polynuclears continue their upward course for a variable time after which they too participate in the decline. Unessential modifications of this type of curve are common but the general characteristics described are usually present. It is true that at times from certain animals a curve differing widely from that described above is obtained, for which no adequate explanation has been offered. The effect produced in dogs by large doses of epinephrine (1 mg. per kg. body weight) is not essentially different from that of the small doses insofar as the white blood cells are concerned. However, in this case, the red cells are increased in number, this increase beginning within fifteen minutes after the alkaloid is given and lasting for about two hours. (Chart I.)

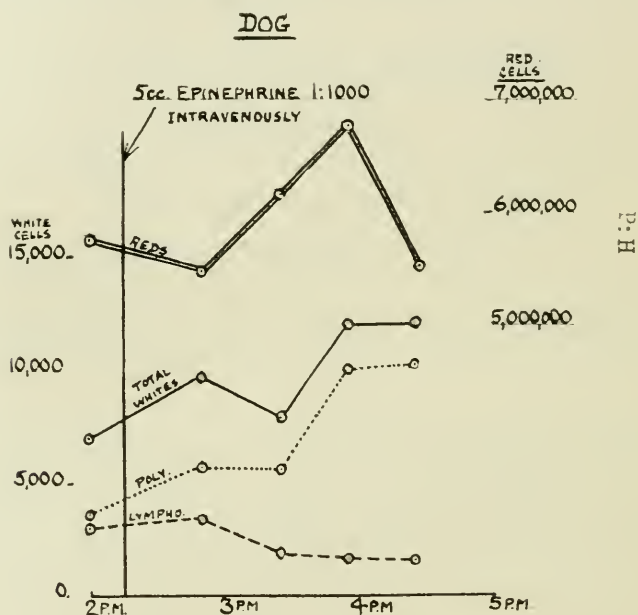


Chart I.—Dog. Effect of administration of large dose of epinephrine.

In all charts the double line indicates the red count with the scale given at the right. Single heavy line is the total leucocyte count with scale given at the left. Dotted line is the polynuclear leucocyte count while the broken line indicates the lymphocyte count. Time is marked on the base line.

On account of the relative delicacy of the reaction of the leucocytes it was thought that it might be possible that they would react to an increase in epinephrine which would be brought about through the injection of the alkaloids named above.

The experiments were carried out essentially as in the work on epinephrine referred to, dogs being used as experimental animals and the blood obtained by a prick on the nose. Some variations in this technic will be mentioned. In the experiments where no operative procedure was necessary, a general anesthetic was not always administered but in a few animals a small injection of morphine was given to keep the animal quiet. Stewart and Rogoff<sup>5</sup> have shown that morphine has no effect upon the output of the

adrenal glands of dogs. In still other dogs, sodium luminal was utilized to keep the animal quiet with rather interesting results as will be shown later. The blood counts were made by the ordinary clinical methods. The spreads were stained with Wright's stain and the differential counts, as well as the counts of the red and white blood cells, were all made by one of us (Lloyd).

The action of strychnine was first studied. As is well known, Stewart and Rogoff<sup>3</sup> have shown that this alkaloid increases to a marked degree the amount of epinephrine secreted by the adrenal glands, even up to 10 or 15 times the normal and this even when the strychnine is given in what may be termed therapeutic doses. In our experiments, the initial dose of strychnine was 1 mg. If after a time, no increase in reflexes was found, an injection of 0.5 mg. was given, which dose was repeated if no marked symptoms

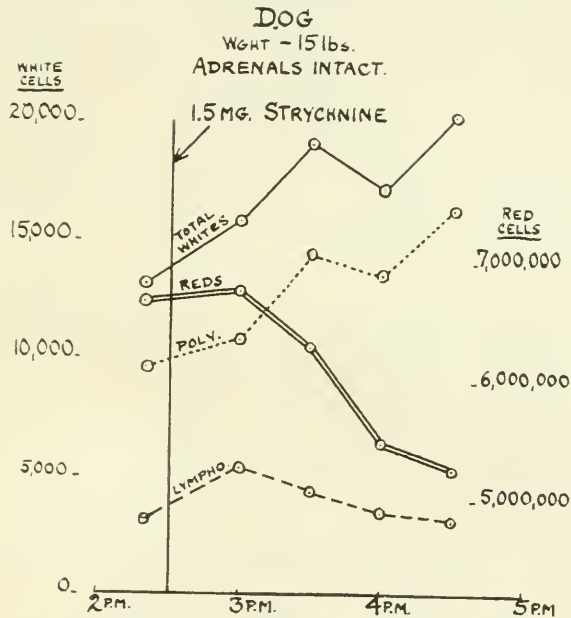


Chart 2.—Dog. Adrenals intact. Subcutaneous administration of 1.5 mg. strychnine. Compare leucocyte curves with those shown in Chart 1. Designation of curves is the same as in Chart 1.

resulted. Increased reflexes usually resulted then and in one dog a total dose of 2 mg. of strychnine was followed by a mild convulsion. The experiments were carried out upon three dogs with uniform results so that the figures and chart of only one need be given. The dog chosen for report was fully anesthetized with chloretone and paraldehyde so that it would be a fair control for the animals which on account of operative procedures had to be anesthetized similarly. The results with or without anesthesia were essentially alike.

The accompanying chart (Chart 2) shows more clearly even than the above figures the general course of a curve which in all its essential details is identical with that which would be given by a small dose of epinephrine.



It is interesting to note that on account of difficulty in getting blood from the nose in two of the counts, cuts were made through the skin over the

TABLE I

MAY 31, 1922	DOG, 15 LBS.		CHLORETONE-PARALDEHYDE		ARTIFICIAL RESPIRATION	
	REDS	WHITES	POLYNUCLEAR	LYMPHOCYTES	MONONUCLEAR	
2:20	6,650,000	13,160	9,700	3,200	260	
2:30	1 mg. strychnine subcutaneously.					
2:40	0.5 mg. strychnine subcutaneously.					
3:00	6,790,000	15,900	10,600	5,100	200	
3:30	6,290,000	19,000	14,500	4,300	200	
4:00	5,480,000	17,000	13,500	3,400	100	
4:30	5,240,000	20,100	16,300	3,300	500	

neck and small arteries opened and the counts made from this actively running blood. This variation in technic produced no distortion of the curve

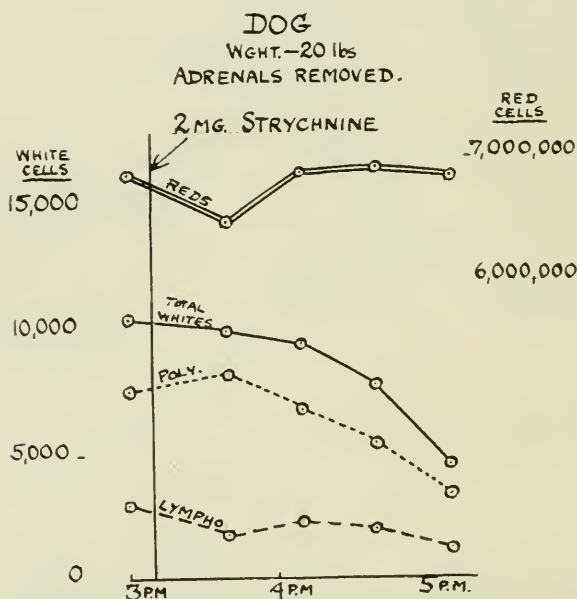


Chart 3.—Dog. Adrenal glands removed. Effect of 2 mg. strychnine. Compare leucocyte curves with those in Chart 2. Note that the red cell curve with these strychninized dogs shows no characteristic course.

which was essentially identical with that obtained from other animals where all the counts were made from capillary blood obtained from the nose. This fact, which was confirmed in other animals under similar conditions, has an important bearing upon the whole question, connected as it is with the theory that a possible unequal distribution of cells might be responsible for the changes in blood count.

In the second series of dogs, both adrenal glands were removed. The abdomen was then closed and the animal allowed to remain quiet for a considerable time in order to permit of proper readjustment of the circulation. Blood counts and spreads were then made. Strychnine was now given by subcutaneous injection as in the control animals, doses of from 1.5 to 2 mg.

being administered. Blood was taken at half hourly intervals and Chart 3 shows a curve obtained under these conditions. Other curves were similar so need not be given nor need the numerical results be cited as they are shown clearly in the chart. The course of the curve is entirely changed. Instead of an increase in leucocytes, there is a decrease. The conclusions are clear. The increase in the number of leucocytes and the change in their relative proportions which are produced in intact normal dogs by strychnine are due to the effect of the alkaloid upon the adrenal glands—in other words, they are epinephrine effects. The number of red cells, however, in these animals is not greatly altered as the amount of epinephrine produced is com-

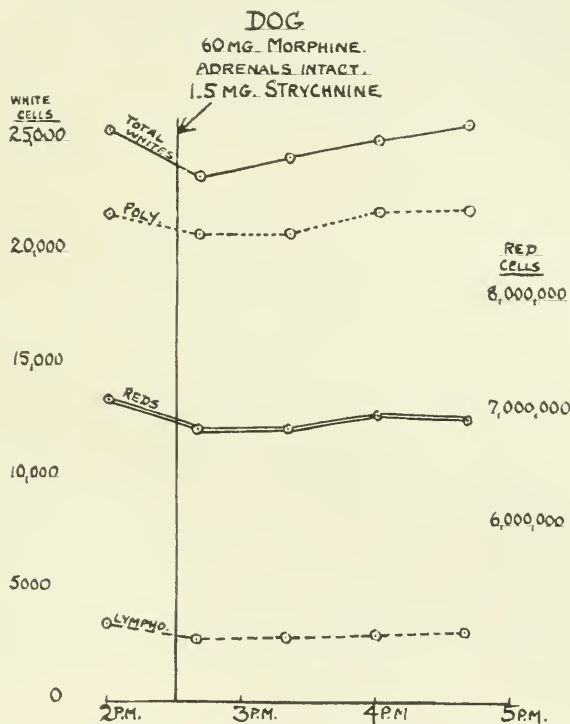


Chart 4.—Dog. Adrenals intact. Preliminary injection of morphine followed after some hours by strychnine 1.5 mg. See text for discussion.

paratively small, while to modify the number of erythrocytes a large amount of epinephrine is required as was pointed out earlier in this paper.

Two or three features encountered in these experiments deserve special mention: first, as to the effect of muscular movements which it might be thought would play a part in modifying the course of the curves. In two dogs each receiving two milligrams of strychnine, convulsions occurred. In neither case was the curve of either red cells or white cells modified. On theoretical grounds marked deviation might have been expected due to a possible squeezing of cells out of the capillaries of the muscles. This did not occur.

Two interesting variations in results were encountered due to the anes-

thetic used. In the first, morphine was employed. Now, Stewart and Rogoff<sup>5</sup> have shown that morphine has no effect upon the epinephrine output in dogs, but that in cats where the morphine has a stimulating effect as shown by the typical excitement, the adrenal glands are stimulated and an increase in epinephrine in the blood is demonstrable. In one of our dogs to which 60 mgs. of morphine were given, wild excitement followed, essentially the same symptoms as are seen in the cat under similar circumstances. In this animal, the reaction to the 1.5 mg. of strychnine as seen in the course of the blood cell curves was much less than in the average normal dog which is depressed by morphine; in fact, the strychnine effects may even be said to be nil as shown in the chart (Chart 4). The explanation doubtless was that this dog's adrenals were already stimulated to increased activity by the morphine and the strychnine effect was not discernible. In other words, this dog reacted to morphine after the manner of cats insofar as its central nervous system was concerned and because of that action its adrenals were stimulated to increased activity.

Another variation in curves was encountered in two dogs. Compelled by the experience just described to give up morphine as an aid in keeping our normal animals quiet, we tried sodium luminal which was recommended as an anesthetic by Drinker.<sup>6</sup> For our purpose, which was simply as a depressant, it was not satisfactory on account of the very severe depression which it produced. The prostration was so marked, the luminal depression of the cord so strong, that the strychnine action was greatly lessened and while epinephrine curves were obtained the changes were not so marked as in animals in which other depressant drugs were employed. In one of these dogs, the total leucocyte count rose from a normal of 14,400 to 16,300 while in the other it rose from 15,000 to 17,000 as compared with increases of 8,000 or 10,000 commonly seen in normal animals. Both morphine and luminal experiments were interesting confirmations of the localization of the strychnine effect upon the adrenals as being upon the central nervous system.<sup>3</sup>

#### CONCLUSIONS

The changes in the total number of leucocytes in the dog and in the relative proportions of the different varieties of white blood cells produced by strychnine are only indirectly due to strychnine but are directly due to the epinephrine which is secreted by the adrenal glands under the influence of strychnine. The epinephrine thus secreted is not in sufficient amount to alter the number of the red blood cells.

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## HISTOLOGICAL STUDY OF MUSCLE DEGENERATION PRODUCED BY LOCAL INJECTIONS OF INSULIN\*

BY D. J. BOWIE, B.Sc., (MED.) AND W. L. ROBINSON, M.B.

**I**N the experimental and clinical use of insulin as a diabetogenic reagent we were struck with the local reactions produced in rabbits and human beings about the site of the inoculation. In the rabbit more particularly one very often found a marked local subcutaneous edema somewhat mucigenous in character and extending for a considerable distance beyond the site of the inoculation.

Histological preparations of this edematous tissue were made and stained with Mallory's eosin and methylene blue, phosphotungstic acid hematoxylin, Van Gieson and Mallory's aniline blue stains. These we studied very carefully, but were unable to find any change in the connective tissue cells nor any abnormal deposits between them of a degenerative character.

The cells were widely separated by an unstainable fluid material. Certain sections, however, which included portions of the neighboring muscles showed degenerative changes going on in some of the superficial muscle fibres. To determine the nature of this degeneration intramuscular injections of insulin were made into a series of rabbits. These were killed at various periods of time after the injection and the nature of the degeneration as well as the sequence of events studied.

EXP. 1.—Rabbit. (1-25-23A) Normal. 1 c.c. of sterile insulin injected into the left sacro-spinalis muscle after shaving and mopping the skin with alcohol. Ten minutes later the muscle tissue removed and fixed in Zenker's fluid. Study of these sections revealed no evidence of muscle degeneration. The stroma was slightly edematous, no evidence of cellular reaction.

EXP. 2.—Rabbit. (1-25-23B) Normal, as in experiment No. 1 injected with 1 c.c. of insulin into the left sacro-spinalis muscle. One half hour later muscle removed. No evidence of oedema in the gross. Sections fixed and stained as before and show some evidence of a beginning degeneration of the muscle fibres, which is characterized by an apparent autolysis of portions of the cytoplasm leaving a fine granular debris. Between these areas are irregular fragments of slightly deeper staining cytoplasm. The longitudinal and transverse striations of these latter fragments of cytoplasm are gone. There is no evidence of an inflammatory reaction. The sarcolemma is intact.

EXP. 3.—Rabbit. (1-25-23C) Normal, as in experiment No. 1 injected with 1 c.c. of insulin into the left sacro-spinalis muscle. One hour later muscle removed. Sections fixed and stained as before show fairly marked degenerative change in groups of the muscle fibres similar to that found in experiment No. 2. The sarcolemma is intact. There is no evidence of inflammatory reaction.

EXP. 4.—Rabbit. (1-4-23B) Normal, injected as in experiment No. 1 with 1 c.c. of insulin into the left sacro-spinalis muscle. Four hours later muscle removed and fixed

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in Zenker's fluid. There was a slight amount of oedema apparent in the gross. Sections stained as before show a pronounced degenerative change in some of the muscle fibres characterized by a wrinkling of the cytoplasm within the sarcolemma into coarse twisted cords and showing a loss of longitudinal and transverse striations.

EXP. 5.—Rabbit. (12-5-22C) Normal, well fed for three days previous to inoculation including glucose in its food. An injection of 1 c.c. of sterile insulin made subcutaneously into the right hind leg. Eight hours later examined. No edema but slight amount of hemorrhage. Neighboring muscle tissue fixed and stained as before show in one or two areas some of the superficial muscle fibres degenerated similarly to experiment No. 4. There is a marked infiltration of the stroma with polymorphonuclear leucocytes. Some of the muscle fibres are infiltrated with these same cells. The sarcolemma is intact.

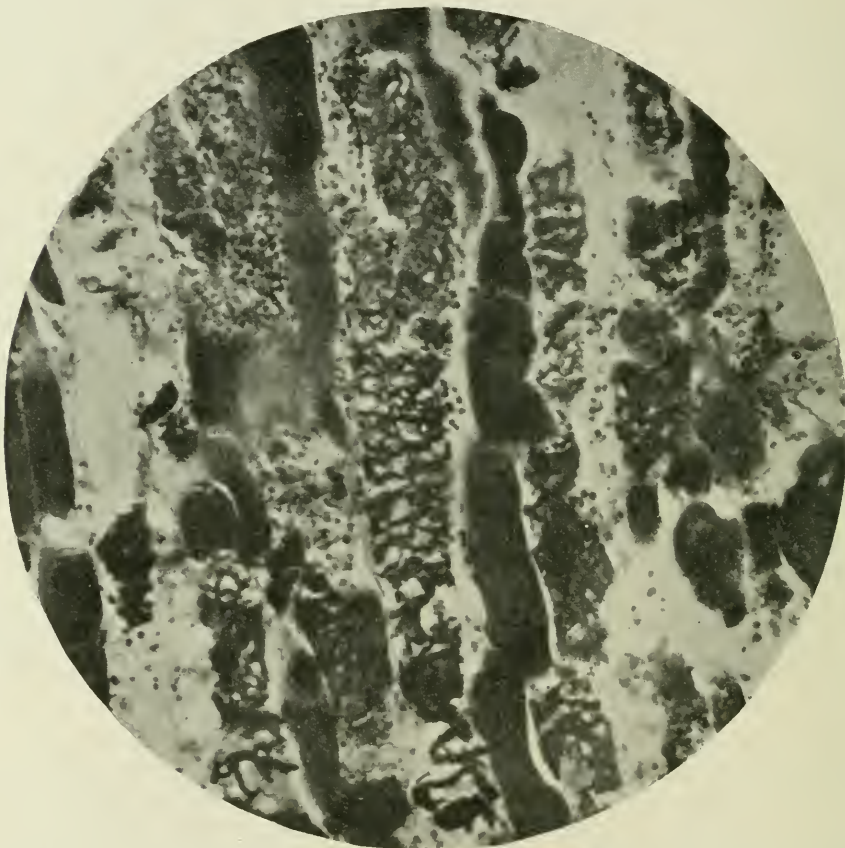


Fig. 1.—Microscopical section of muscle near site of injection of insulin showing characteristic changes especially in Experiments 4 and 6.

EXP. 6.—Rabbit. (0-26-22) Normal, injected subcutaneously into the back with 4 c.c. of insulin. Five hours later given another injection of 4 c.c. subcutaneously into the back. Animal died twelve hours later. Marked subcutaneous edema over the back. Superficial muscles of this area fixed and stained as before show a moderate diffuse lymphocytic infiltration of the external sheath of the muscle. About one third of the muscle fibres show degeneration as described in experiment No. 4 but more extensive. Cross section of degenerated fibres show some wrinklins of cytoplasm into irregular coils with no directional tendency. The muscle fibres are affected in a very irregular manner. Sometimes two or three fibres together are degenerated, in other areas just isolated fibres show this change. Usually just a portion of any one fiber appears degenerated.

In order to determine whether this degeneration is characteristic of insulin or some toxin in its preparation a series of rabbits were injected intramuscularly with saline, glucose and adrenalin solutions as well as insulin. These were checked one against the other in the same rabbit by carefully shaving and mopping the skin with alcohol and injecting one solution into the sacrospinalis muscle and one of the other solutions in the same muscle on the opposite side. Eight hours was allowed in each case, as this gave plenty of time for the degeneration to reach its maximum. The pieces of muscle tissue removed from the site of inoculation were fixed in 10 per cent formalin, embedded in paraffin and stained with hematoxylin and eosin.

EXP. 7.—Rabbit (2-28-23A) Normal (A) 1 c.c. sterile isotonic glucose injected into left sacro-spinalis muscle. Microscopic section shows slight amount of muscle degeneration immediately about the needle puncture, probably traumatic. The muscle degeneration is apparently the same as described in experiment No. 4.

(B) One c.c. sterile hypertonic glucose injected into right sacro-spinalis muscle. Degeneration the same but much more extensive.

EXP. 8.—Rabbit. (2-28-23B) Normal. (A) One c.c. sterile hypertonic glucose solution injected into left sacro-spinalis muscle. Muscle degeneration is very extensive and marked, similar to (B) in experiment No. 7.

(B) One c.c. sterile insulin injected into right sacro-spinalis muscle. Muscle degeneration similar to, but more extensive than that of the opposite side.

EXP. 9.—Rabbit. (2-28-23C) Normal (A) One c.c. sterile insulin injected into left sacro-spinalis muscle marked and extensive muscle degeneration similar to (B) Exp. 8.

(B) One c.c. of 1-10,000 sterile adrenalin solution injected into right sacro-spinalis muscle. Very slight amount of muscle degeneration but similar to that described in experiment No. 4.

EXP. 10.—Rabbit. (2-28-23) Normal. (A) One c.c. sterile isotonic saline injected into left sacro-spinalis muscle. Small localized area of muscle degeneration about puncture wound, probably traumatic.

(B) One c.c. sterile hypertonic saline solution injected into the right sacro-spinalis muscle. Moderate amount of muscle degeneration similar to above experiments.

Exp. 11.—Rabbit. (3-7-23A) Normal. (A) One c.c. sterile 3 per cent hypertonic saline solution injected into left sacro-spinalis muscle. Slight amount of muscle degeneration confined pretty well to needle puncture wound. Partly or wholly traumatic.

(B) One c.c. of a 6 per cent hypertonic sterile saline solution injected into the right sacro-spinalis muscle. Muscle degeneration a little more extensive than that found on the opposite side.

EXP. 12.—Rabbit. (3-7-23B) Normal. (A) One c.c. sterile 1-1,000 adrenalin solution injected into left sacro-spinalis muscle. Muscle degeneration is very extensive and with edema of the stroma.

(B) One c.c. of a 1-10,000 sterile solution of adrenalin injected into the right sacro-spinalis muscle. Muscle degeneration a little more marked and extensive than the opposite side.

It was expected when this work was undertaken that insulin would be found to have a marked local effect on subcutaneous tissue and in muscle because of the edematous condition so often observed in animals treated with insulin and sugar.<sup>1</sup> As the foregoing observations show it was indeed found that intra-muscular injections called forth near the site of injection a very

marked degeneration of the muscular fibres with leucocytic invasion. This same type of lesion, however, was found to be caused by other agencies such, for example, as hypertonic glucose solution. Isotonic saline, on the other hand, as a rule produced only slight localized areas of degeneration although hypertonic solutions had an effect like that of insulin.

The accounts of the lesions indicated by these studies following intramuscular injections explain probably the considerable pain and discomfort that is not uncommonly complained of and it may be pointed out that if the lesions can be produced by hypertonic saline it is altogether likely that they might become quite extensive when various therapeutic agents are employed.

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### THE EFFECT OF PHENOL IN THE ESTIMATION OF REDUCING SUGARS BY THE PICRAMIC ACID METHODS\*

BY HENRY C. SWEANY, CHICAGO, ILL.

A FEW years ago Lewis and Benedict<sup>1</sup> devised a colorimetric method for the estimation of blood sugar based upon the reduction of picric acid to picramic acid by an alkaline solution of the glucose present in blood. The method consists of taking 2 c.c. of blood in 5 c.c. of water, adding 15 c.c. of a saturated solution of picric acid and making the final volume up to 25 c.c. with water, taking 8 c.c. of this filtrate in a test-tube, adding 2 c.c. of a saturated solution of picric acid and 1 c.c. of 10 per cent sodium carbonate and evaporating the mixture to dryness over a free flame. The dry crystals are dissolved in 3 c.c. of water with heat, made up to 10 c.c., filtered and read in a colorimeter against a standard solution of picramic acid. The original method with various modifications has been very generally accepted among clinicians and medical research workers as it is relatively simple, rapid and presumably accurate, if properly controlled. Other factors being ignored, the accuracy depends, of course, upon the most exact adherence to the empirical details of the procedure. The most important factors are, (a) the H-ion concentration at the time of precipitation of the proteins by the picric acid, (b) the concentration of the acid remaining in the filtrate, (c) the OH-ion concentration at the time of heating, (d) the period of heating, and (e) the temperature of heating. The only variable should be the glucose concentration. All other factors should be carefully controlled and values for the various concentrations of glucose carefully determined, as in the various copper sulphate reduc-

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tion methods. Some of these factors have been quite satisfactorily controlled by various workers,<sup>2, 3, 4</sup> and are taken into account in Benedict's latest modification of the Lewis-Benedict method.

The method has been criticized<sup>2, 3, 4, 5</sup> in that the reduction of pieric acid to picramic acid may also be brought about by creatinine, uric acid, polyphenols, glycuronic acid, aldehydes, ketones, etc. However, the greatest portion of these reducing substances is probably eliminated by the use of proper concentration of pieric acid or the proper dilution of the filtrate. Still it seems reasonable to suspect that at least traces of these substances remain and that a summation of effects of all these reducing substances produce a marked error even in normal subjects. Any reagent that is as easily reduced as alkaline pierate must be rigidly controlled. Therefore, if the method is to be reliable in the determination of sugar in such a complex mixture as blood, especially in pathologic specimens in which unknown reducing agents may be present, it should be thoroughly tested against other standard methods or by some other acceptable means. This, it seems, has not been done satisfactorily.

Aside from the direct effect of these reducing substances, the author has noticed a combined effect produced by certain substances; the most notable substance producing this effect and the only one studied to any extent is phenol, although the polyphenols, cresols, conjugated phenols, ether, and various other substances have been found to cause apparently similar interference. For example, a concentration of phenol of about 1 part in 4000 or 2 mg. in 8 c.c. of blood filtrate will cause a trebling of the color reading, although phenol alone will cause no reduction of pieric acid, neither will it cause any change in color when added *after the full color has been developed*. It must be present while the glucose molecule is in a stage of active disintegration. This effect of phenol and allied substances may be responsible for many of the discrepancies found in these methods. While the results appear not abnormal in nonpathologic specimens it is possible that the high results of blood sugar found in certain hospital patients by Myers<sup>2</sup> and the increase above the results obtained by the Folin method by Host and Hatlehoel<sup>6</sup> and Wesselow<sup>7</sup> may be due to a similar interference.

There are, therefore, two basic faults with the methods in question: first, the direct effect of pierate reducing substances other than glucose in the blood and second, the combined effect of substances like phenol which produce excess color in the presence of glucose. In the absence of interfering substances the question of time and temperature is not so important if a standard is *run exactly parallel to the test*. In that way the same oxidation and reduction is taking place in both mixtures and a comparative result though incomplete in both will be fairly accurate. In the presence of interfering substances, however, there is no correspondence between the standard and the unknown as we have determined by experiment. Therefore, it is not accurate to compare a blood mixture filtrate with an aqueous solution of glucose. To use an empirical color standard or even a standard picramic acid solution will run the risk of making grave errors, because the variation



of conditions from day to day varies the oxidation process of the glucose to a marked degree.

True, some of these criticisms may be applied to all blood sugar methods. It was with the idea in view to work out a rational system of comparison of the various methods and to determine if possible the extent to which phenols and other similar substances interfere with the tests that prompted this work.

The reagents used were the purest possible to secure. The picric acid was purified according to Folin and Doisy's method;<sup>8</sup> the glucose was the snow white anhydrous product of the Specials Chemical Co., and the other reagents were equally as well chosen. Impure picric acid causes an abnormal increase in color, a fact observed by Rhode,<sup>9</sup> and others. Undoubtedly the presence of traces of unchanged phenol, mono-, or dinitro-phenol, is responsible for this additional color.

Before making any tests the methods were checked with known concentrations of glucose for the various effects that might be produced by changes in concentration of alkali and picric acid; by changes in time of heating; and by changes in volume of the heated mixture. In general, both methods were sufficiently accurate in all these details to preclude any error in aqueous solutions (except the usual experimental error) when the technic outlined above was followed correctly. The results of these experiments will not be repeated here. Addis and Shevky<sup>10</sup> have covered these points thoroughly. Suffice it to say that sugar could be recovered within 2 to 3 per cent in aqueous solutions.

#### EXPERIMENTAL

In order to establish any defect in these various methods it was necessary to carry out a series of experiments: first, in aqueous solutions in which all factors could be controlled; and second, in blood mixtures in which various unknown reducing agents might be present. The Lewis and Benedict<sup>1</sup> method and Benedict's modification<sup>4</sup> were used to represent the picramic acid methods while the Shaffer-Bertrand<sup>11</sup> method was used as a check. This method was not used as an absolute check so much as a comparative method.

The technic of the new Benedict method is in brief as follows: 2 c.c. of whole blood are laked with 2 c.c. of water and made up to 25 c.c. gradually and with gentle rotation with sodium picrate solution. This is filtered and to 8 c.c. of the filtrate is added 1 c.c. of 20 per cent sodium carbonate solution. After mixing, this is heated for ten minutes in a water bath at 100° C. and read against a standard similarly prepared. The sodium picrate for this method is prepared by dissolving 36 gm. of purified picric acid in water, with the aid of exactly 16 gm. of sodium hydroxide, the solution is filtered, cooled and made up to 1000 c.c. It should have an acidity of 0.05 to 0.04 normal.

Shaffer's Method of Blood Sugar Analysis is as follows:

Draw 5 c.c. of blood in 25 c.c. water, heat in beaker or flask just to boiling, rotating flask gently. Add a few drops of dilute acetic acid to the hot liquid to produce visible coagulation. Five c.c. colloidal iron solution (Merek's Dialyzed) is added, the mixture is well shaken; after which about 0.2 gm.

of powdered  $\text{Na}_2\text{SO}_4$  is added and the mixture again shaken for 10 seconds. The liquid is then poured into a 50 c.c. centrifuge tube and centrifugated for 2 minutes. The liquid is then poured off through a small filter and 21 c.c. of the water clear liquid equivalent to 3 c.c. of blood are measured into another 50 c.c. tube. Twenty-one c.c. of mixed Fehling's is added and the tube immersed in boiling water for ten minutes when the contents is again centrifugated, washed and the  $\text{Cu}_2\text{O}$  is determined by Bertrand's titration.

After working with the methods for some time, it was found expedient to adopt a more rigid technic than is called for by any of the described methods before consistent results could be obtained. For example, the blood

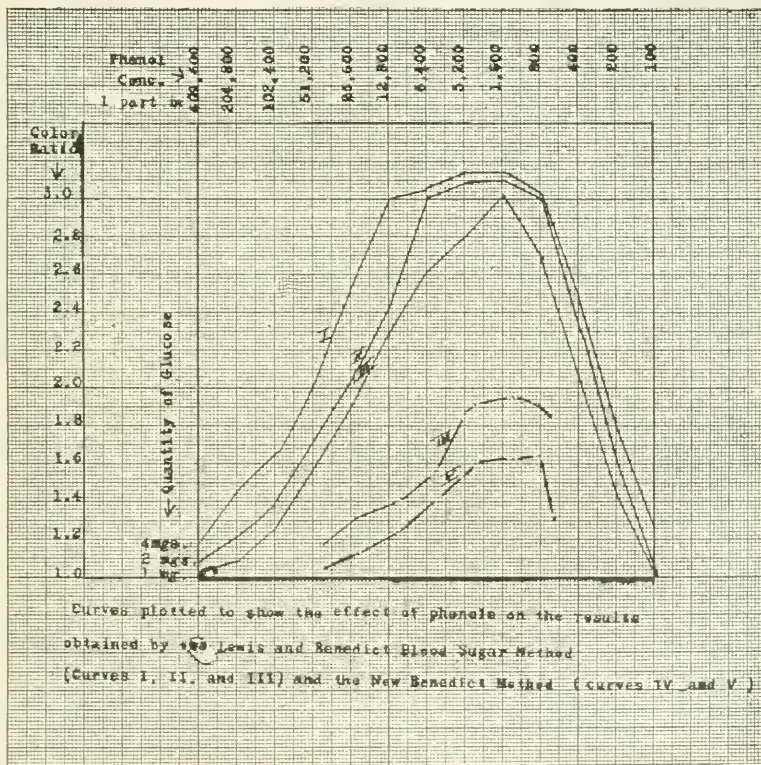


Chart 1.

was laked and precipitated in approximately two hours after drawing by exactly the same procedure each time; the precipitate was collected upon a filter within 30 minutes of the time of precipitation, and the filtrate was heated and analyzed on the same day. The tubes were well shaken before placing in the bath and the reagents were added in the same order each time.

After a cursory trial of various substances that might cause an intensification of color, it was found that most of the phenols as well as a large number of other compounds such as uric acid, creatinine, etc., gave an exaggerated reading. Phenol and potassium phenyl sulphate were then selected for a more detailed study.

A series of experiments was performed in which the phenol concentration was varied with 0.64, 1, 2, and 5 milligrams of glucose respectively. The final volume, the concentration of alkali and the picric acid, the period and temperature of heating were kept constant. The results obtained by the old Lewis and Benedict method are given in Table I and plotted in Chart 1.

TABLE I

PHENOL CONCENTRATION ONE PART IN—	409600	204800	102400	51200	25600	12800
(1mg. ( dextrose	1.02	1.08	1.26	1.57	1.90	2.28
Color (2mg. ( dextrose	1.08	1.21	1.37	1.73	2.04	2.43
(4mg. ( dextrose	1.18	1.45	1.65	2.01	2.53	2.98
continued—	6400	3200	1600	800	400	200
	2.60	2.80	3.02	2.70	2.07	1.44
	3.00	3.08	3.10	3.00	2.31	1.61
	3.04	3.13	3.14	3.02	2.48	1.82
					1.82	1.28

Chart 1 shows clearly the effect of phenol on the color formation. At about one part in 4000 of phenol the color is three-fold. This was repeated by the new Benedict method with one concentration of sugar, viz., 0.64 mg. in 10 c.c. of solution. The results are shown in Table II and Curve IV on Chart 1.

TABLE II

0.64 MG. DEXTROSE						
PHENOL CONCENTRATION ONE PART IN—	40000	20000	10000	5000	3333	
(Phenol	1.19	1.31	1.35	1.56	1.87	
(Potassium	1.05	1.12	1.24	1.42	1.52	
Ratio (Phenyl						
(Sulphate						
continued—	2000	1333	1000	750		
	1.91	1.95	1.91	1.85		
	1.60	1.62	1.64	1.30		

Table II and Curve V on Chart 1 represent the results obtained with varying concentrations of potassium phenyl sulphate on 0.64 mg. glucose. The color change in this experiment reached only twice the normal instead of three times, as in the original method. By increasing the alkali as the phenol increases, the color change can be kept from decreasing after it has reached its maximum. The decrease in OH-ion concentration, therefore, accounts for the drop in the curves on the right side of the Chart.

In order to study the effect that such agents as phenol might have on these methods when applied to blood, experiments were accordingly performed on rabbits and dogs. Thus, a rabbit was bled 20 c.c. from the heart, then injected with 10 c.c. of a 2.5 per cent phenol solution and again bled 20 c.c. within 5 minutes. The two samples were analyzed for blood sugar



by the new Benedict method and by Shaffer's method. The results are found in Table III.

TABLE III

	BEFORE	AFTER
Lewis and Benedict	125	465
Shaffer	116	220

These results show a decided "false" reading in the Benedict method but also show a doubling of the value found in the Shaffer method. This could be due to the effect of the drug on the glycogenetic ferment of the liver, to "fear hyperglycemia," or some allied process. However, it is impossible by such explanations to account for the great variation between the two methods in the sample of blood taken after the injection. This we assume is caused by some interfering substance such as the phenol that was injected. To attempt to eliminate this actual hyperglycemia and to establish more clearly the effect of an interfering substance such as phenol, the work was repeated on a dog, because by proper manipulation the dog is not so susceptible to "fear hyperglycemia," and 20 c.c. of 10 per cent potassium phenyl sulphate was substituted for the phenol, because phenol may set up an irritation that will liberate blood sugar. The results are tabulated in Table IV.

TABLE IV

	BENEDICT'S METHOD	SHAFFER'S METHOD
Before Injection	0.080 mg. sugar	.069 mg. sugar
After Injection	0.110 mg. sugar	.072 mg. sugar
Difference	0.030 mg. sugar	.003 mg. sugar

This shows very little change in the results before and after injection in the Shaffer method but a definite increase by the Benedict method. This experiment demonstrates clearly the unfavorable effect of such substances with the Benedict method. In a subsequent paper a more complete comparison will be made by analyzing a large number of normal and pathologic samples of blood, using the various blood sugar methods.

#### DISCUSSION

The exact mechanism of this reaction is rather difficult to understand. Phenol itself will not produce any color change with alkaline picrate, nor will it modify the color if added *after* the full color has been developed in the alkaline picrate by the glucose only present when the glucose molecule is undergoing disintegration does it affect the results. The interference of phenol might be accounted for in the following manner: first, the glucose and phenol may unite, and cause increased reduction of picric acid. However, should they unite in the normal manner for phenols and aldehydes, the aldehyde group would be removed and thus there should be a lessening in the amount of color; second, the glucose-phenol combination may unite with picric acid to form a more highly colored addition compound, but this seems



unlikely; third, the phenol may react with the decomposition products of glucose to give a colored product or one which reduces the picric acid to picramic acid. The latter seems to be the most plausible explanation. Nef<sup>12</sup> has shown that under different conditions different quantities of end products, as well as different substances of glucose oxidation will result. Gaud<sup>13</sup> also shows that varying the alkali will cause a vast difference in the products of glucose oxidation. In any event, the process is not so fixed and stable as is commonly thought. It seems reasonable, therefore, to suppose that the phenol enters into the reaction in a manner that may cause a more complete oxidation of glucose fragments or may be itself oxidized to a substance like pyrochtechin (meta-dioxy-phenol) which in turn reduces more picric acid.

From a practical standpoint, however, the most important fact to consider is the true worth of these methods clinically. Especially is this true in pathologic specimens in which a true result is imperative to sound diagnosis and treatment. There is no reason to believe that phenol is the only substance that can interfere with the reaction. In fact there are good reasons to believe that there are many substances that will have a similar effect.

#### SUMMARY

1. It has been shown that the presence of phenol interferes with the determination of sugar by the Lewis and Benedict and the new Benedict methods, giving a two- to three-fold color when one part of phenol is present in 4000 parts of solution of phenol.

2. In the determination of blood sugar, the phenols present in normal blood cause a 10 per cent error by the Lewis and Benedict method, but practically a negligible error by the new Benedict method.

3. For a similar reason where picramic acid methods are applied to the determination of sugar in the urine without the elimination of phenols, polyphenols, etc., there will result an error of 20-50 per cent.

4. No picramic acid method can be used for the determination of sugar in solutions preserved by phenols and many related coal-tar products.

5. A possible explanation of the phenol interference has been suggested.

The author wishes to express sincere appreciation to Dr. F. C. Koch, under whose direction part of this work was carried out, for valuable assistance.

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## CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

BY A. LEVINSON, M.D., CHICAGO, ILL.

### URINE

(Continued from page 537.)

**COLLECTION OF URINE.**—From children over two years of age, urine can be obtained by having the child urinate into a vessel. From male infants urine may be collected by attaching a wide-mouthed bottle or test tube or a portion of a rubber glove over the genital organ, fastening it in place by means of adhesive plaster attached to the pubic area (Fig. 20).

From female infants, urine may be obtained by attaching a short wide-mouthed bottle over the vulva, fastening it in place by means of adhesive plaster pasted to the pubic area. Bird seed vessels and folded glass ink stands may also be used.

**CATHETERIZATION.**—Catheterization should be avoided as much as possible, as it traumatizes the genitals and may cause infection of the genitourinary tract. When no specimen of urine can be obtained any other way, or when a sterile specimen is desired for bacteriologic examination catheterization has to be employed.

*Technic of Catheterization.*—The infant is placed in the recumbent posture and the legs are spread apart. The genital organs are washed with cotton saturated in 1 per cent lysol or in alcohol, or in 1 to 5,000 bichloride of mercury solution. A sterilized metal catheter, a rubber catheter (10 French) or a glass catheter, the latter being the least desirable, is introduced for a distance of  $1\frac{1}{2}$  to  $1\frac{2}{3}$  of an inch in the female infant and 1 to  $1\frac{1}{2}$  inches in the male infant. An eustachian tube catheter (Fig. 21) is the ideal catheter for the female infant. A receptacle is held at the distal end of the catheter for the collection of the urine (Fig. 22).

**COLOR.**—In order to obtain any information from the color of the urine in infants and children, one has to keep in mind that the color of the urine changes with age, with the type of food, and with the amount of fluid consumed.

In the newly born, the urine is concentrated and often is of brick red color, supposedly due to a uric acid infarct present in the kidneys of the newly born. All through infancy, the urine is straw-colored. It becomes concentrated and assumes a light red color in diarrhea and in febrile conditions.

In older children, the urine is also straw-colored, but may become more concentrated, after the ingestion of food. At times a brick red sediment, made up of uric acid, settles at the bottom of the container after the consummation of a large amount of meat. In all febrile conditions the urine is concentrated and may assume a dark red color.

In catarrhal jaundice, as well as in icterus neonatorum the urine is greenish red in color. In hemorrhagic nephritis the urine is dark red, and at times looks like pure blood. In tuberculosis of the kidney, in calculus of the kidney, ureters or bladder the color may also be bloody. In abscesses of the pelvis of the kidney and in pyelitis the urine may be very turbid.

**SPECIFIC GRAVITY.**—For clinical purposes, the ordinary urinometer furnishes valuable information. The specific gravity in the urine of the newborn is quite high, varying between 1.020 and 1.025. During infancy, the specific gravity varies between 1.010 and 1.018. It usually changes with the time of the day and with the intake of food. A fixed specific gravity speaks for a pathologic condition. A persistently high specific gravity speaks for diabetes or acute nephritis. A persistently low specific gravity points to chronic nephritis. (See Mosenthal test.)

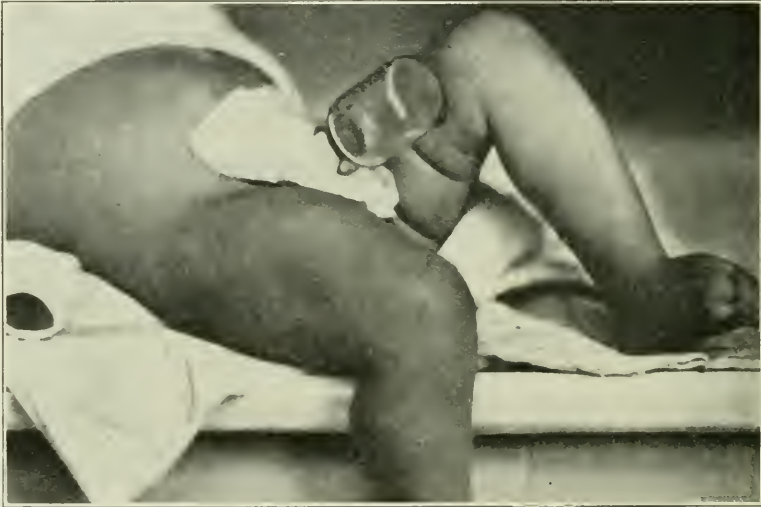


Fig. 20.—Method of collecting single specimen of urine.

**REACTION.**—For clinical purposes, litmus paper may be used for the determination of the reaction of urine. Immediately after the urine has been voided, it is acid in reaction. On standing several hours it assumes a neutral or alkaline reaction. Urine is often alkaline one or two hours after ingestion of food. This is known as the "alkaline tide." Neutral or alkaline reaction in a fresh specimen, not following a meal, indicates stagnation of the urine in the bladder, as happens in paralysis of the bladder.

In the administration of alkalis for the treatment of pyelitis, the reaction of urine may serve as a guide as to how much alkalis to give. As soon as the reaction of the urine becomes alkaline, the alkalis should be discontinued. Table IX from Palmer and Van Slyke may be utilized for the determination of the amount of alkali to be administered in order to turn the urine alkaline.

**ALBUMIN.**—The two most simple tests for albumin in urine are: The com-

bined heat and acetic test and the nitric acid test. The combined heat and acetic acid test is done as follows:

The upper part of a tall column of urine in a test tube is heated. A few drops of 5 per cent acetic acid are added, and the urine is examined for a precipitate or turbidity by transmitted light against a dark background. A precipitate indicates the presence of albumin. Phosphates also produce a precipitate on heating, but the precipitate disappears on the addition of acetic acid, whereas an albumin coagulum will usually be intensified unless a considerable excess of acid is used.

The nitric acid test is carried out by letting a few drops of nitric acid run down from a pipette on the inner wall of a test tube containing 2 to 5 c.c. of urine. If albumin is present a white ring will appear at the contact of the nitric acid and the urine.



Fig. 21.—Eustachian tube which may be used as female urethral catheter.



Fig. 22.—Catheterization of a female infant.

TABLE IX

SODIUM BICARBONATE PER KILO BODY WEIGHT REQUIRED TO TURN URINE ALKALINE	MINIMUM PLASMA BICARBONATE CO <sub>2</sub> INDICATED	MAXIMUM DEGREE OF ACIDOSIS INDICATED
gm.	vol. per cent.	
0.0–0.5	55	None
0.5–0.8	55–40	Mild
0.8–1.1	40–30	Moderate to severe
Over 1.1	Below 30	Severe

When several specimens of urine are to be examined, the nitric acid test can be simplified still further by having 5 to 6 c.c. of nitric acid in a test tube and by drawing up a small quantity of urine with a glass tube by capillary traction and introducing the lower end of the tube into the nitric acid. If



albumin is present, a ring will form in the small tube on contact of the urine and acid.

*Quantitative Albumin.*—An Esbach tube used for this purpose is filled with urine to the mark “U” and the reagent is added to the mark “R.” The reagent used is either that of Esbach, or that of Tsuehiya.

The Esbach reagent consists of a solution of 10 grams of picric acid, and 20 grams of citric acid in 1 liter of boiling water cooled off before using.

The Tsuehiya reagent consists of 1.5 gm. of phosphotungstic acid, 5 c.c. of concentrated hydrochloric acid and 95 per cent alcohol to make up to 100 c.c.

The Esbach tube is corked and inverted several times to insure thorough mixing, and allowed to stand in a vertical position for 24 hours. The height of precipitate which settles to the bottom is read on the scale marked on the tube. The figure obtained gives the quantity of albumin in grams per liter of urine. From this the amount of albumin in the given 24 hour specimen is readily calculated. If the urine shows a heavy precipitate by the qualitative albumin test, it should be diluted 1 to 10 before the quantitative albumin reagent is added, and the final reading multiplied by 10.

*Interpretation.*—A negative albumin test is valuable in excluding nephritis. A positive albumin test does not necessarily mean the presence of a nephritis, as all infectious diseases may show a temporary albuminuria. There is also a condition known as orthostatic albuminuria, where albumin is found in the urine of nonnephritic patients when the patient is up and around, and which disappears when the patient is in bed. It is wise, however, to keep all patients showing albuminuria under observation, so as to not overlook a nephritis. Quantitative albumin determination throws light on the amount of destruction going on in the kidneys, or the amount of protein of the blood permeated through the kidneys.

*QUALITATIVE DETERMINATION OF SUGAR.*—Haines', Fehling's and Benedict's methods are commonly employed. Fehling's method has the disadvantage of consisting of two separate solutions. Haines' and Benedict's reagents are therefore most commonly used. Haines' solution is made by dissolving 8.314 gms. of copper sulphate in 400 c.c. of water, adding 40 c.c. of glycerin, and then 500 c.c. of 5 per cent potassium hydroxide. Two to 3 c.c. of Haines' solution is placed in a test tube and boiled gently. No precipitate should form. Several drops of urine are now added and the mixture brought to a boil. A yellow-red precipitate of copper oxide forms immediately or in a few minutes if sugar is present in the urine.

*QUALITATIVE DETERMINATION OF SUGAR.*—Haines', Fehling's and Benedict's methods may be used, the Benedict method being most frequently used.

The Benedict reagent is prepared as follows:

One hundred grams of anhydrous sodium carbonate, 200 gm. of sodium citrate, and 125 gm. of potassium sulphocyanate are dissolved over a flame in about 800 c.c. of water and filtered. In another vessel is dissolved 18 gm. of crystallized copper sulphate in 100 c.c. of water. The copper sulphate solution is now slowly added to the filtrate with constant stirring.

Twenty-five c.c. of this reagent will reduce 0.050 gm. glucose. It may be

necessary to titrate the Benedict's solution to make certain that it will reduce 0.050 gm. of glucose. For the examination of the urine the following procedure is employed: Twenty-five c.c. of the Benedict reagent is measured into a porcelain evaporating dish. 5 or 10 gms. of solid anhydrous sodium carbonate is added. A small amount of talcum powder may be added to prevent bumping. This is now heated over a flame until the carbonate is dissolved. From a burette, urine, diluted 1 to 10, is run in until the blue color disappears, this being the end point. The reagent should be kept boiling during the titration.

To obtain the percentage of sugar in the urine, the following formula is used:

0.050 (amount of glucose reduced by quantity of reagent taken) is divided by X (the number of c.c. of diluted urine taken), times 1000 c.c. ( $100 \times 10$  which was the dilution of urine) or  $\frac{0.050}{X \times 1000}$

*Interpretation.*—The reduction tests for sugar are at times misleading. Often a reduction is obtained with Haines' or Benedict's solution that is grayish in color. This is due to the ingestion of salicylates or acetylsalicylic acid and must not be taken to indicate the presence of sugar in the urine. When in doubt, the fermentation test or the phenylhydrazine test should be employed. Ingestion of large quantities of sugar will give a temporary positive sugar test. When sugar is present in urine, the diagnosis of diabetes should not be made until several specimens have been examined and until the food factor (ingestion of large amounts of sugar) has been excluded, and until the sugar in the blood has been determined and found to be higher than normal. The latter will exclude renal diabetes.

**ACETONE.**—To a few c.c. of urine are added a few drops of a freshly prepared solution of sodium nitroprusside and a few drops to  $\frac{1}{2}$  c.c. of glacial acetic acid. A small quantity of ammonium hydroxide is superimposed on it. A violet contact ring appears and becomes intensified in a minute or two if acetone is present in the urine.

**DIACETIC ACID.**—Gerhardt's test is used. A few drops of 10 per cent ferric chloride are added to the urine. If a precipitate of phosphate forms, ferric chloride is added until no more precipitate forms, and the solution is filtered. In the presence of diacetic acid a Burgundy-red color appears. The color becomes weaker, or disappears on heating; if caused by drugs, like salicylic acid, aspirin, salol, diuretin and phenacetin, the color persists.

Acetone in urine may be due to diabetes. In children, however, it is often present in urine in cases of prolonged starvation. Too much weight must therefore not be placed on the presence of acetone, except as an indication of starvation.

Indican in the urine was at one time supposed to be an indication of the amount of putrefaction in the bowels. This, however, does not seem to be true.

**MICROSCOPIC EXAMINATION.**—In acute nephritis casts and cells are present in the urine in such large amounts that they may be detected under the microscope in an uncentrifuged specimen. In less severe conditions the urine should be centrifuged, the supernatant fluid poured off, and the sediment examined on a clean slide under the microscope. The sediment should be

spread in a thin layer, otherwise the examination is unsuccessful. A cover glass over the slide helps to even the layer of sediment.

**CASTS.**—The presence of casts in urine indicates a destructive process going on in the kidneys. Granular and hyaline casts are most important in the diagnosis of nephritis. Epithelial casts do not justify the diagnosis of nephritis. Care must be taken to differentiate between casts, crystals and debris.

**CELLS.**—The presence of many red cells in the urine indicates a hemorrhage, or at least a marked irritation of the kidney. Such is the case in tuberculosis of the kidney and in hemorrhagic nephritis. An occasional white cell in the microscopic field is present even in normal urine. In pyelitis the white cells are present in large numbers, and are usually clumped.

In examination for tubercle bacilli the urine, which has preferably been standing in the refrigerator for 12 to 24 hours, is centrifuged for  $\frac{1}{2}$  to 1 hour and the sediment is stained by the Ziehl-Neelsen method. Enough alcohol should be used for decolorization in order to exclude smegma bacilli.

**CRYSTALS.**—Uric acid crystals are of no significance. Their presence does not indicate an increased uric acid content of the urine.

Urates, calcium oxalate, and calcium carbonate are of no significance.

Triple phosphates, ammonium, magnesium and calcium phosphate are of significance. They occur in alkaline urine, and usually signify stagnation. The conditions are observed in paraplegia, chronic pyelitis, and chronic cystitis.

**BLOOD.**—In addition to the microscopic method, a chemical method may be used. The guaiac test is most often employed. The technic is as follows:

Freshly prepared tincture of guaiac (small amount of guaiac dissolved in a few c.c. of absolute alcohol) is added to 5 c.c. of the urine until the urine becomes turbid. Hydrogen peroxide is now added, drop by drop, until a blue color is produced, or until 2 c.c. has been added. If the urine contains either blood or pus, a deep blue color results.

**BILE.**—A few c.c. of urine is superimposed on 1 to 2 c.c. of concentrated nitric acid. A green ring, which may change to blue, violet, red or yellow, forms at the line of contact.

**QUANTITATIVE TESTS.**—The following are most important quantitative tests:

Amount in 24 hours

Albumin and sugar when present (described above)

Total nitrogen

Urea nitrogen

Creatinine

Chlorides

**AMOUNT.**—The amount of urine excreted in 24 hours is by itself a good kidney function test. An increased amount of urine is present in diabetes insipidus, and in chronic interstitial nephritis. The urine is decreased in amount in all febrile diseases, and in cardiorenal affections. As an arbitrary standard, 12 ounces should be considered the minimum amount of urine to be voided in 24 hours by a child between 6 to 12 years of age, and 16 ounces in 24 hours by a child above 12 years of age. The intake of fluid should be



measured in conjunction with the urine output, as the latter is naturally influenced by the former.

In administering digitalis, the 24 hour urine output is an important guide in the therapy. If the urine is increased in amount after the administration of the drug, the drug is performing its function. If the urine output is not increased by the medication, the digitalis is not producing its effect on the heart, or the decreased elimination of urine may be due to renal disturbances, instead of cardiac, for digitalis has no, or only slight, diuretic effect in renal conditions.

**TOTAL NITROGEN.**—The total nitrogen in urine varies with the amount of protein ingested. On a mixed diet the total nitrogen output varies between 4 to 7 gm. in 24 hours, in normal children five to ten years of age. This amount is lessened in nephritis with retention, and in uremia.

**Method of Determination.**—The urine is diluted according to its specific gravity.

SPECIFIC GRAVITY	DILUTION
1.010 or below .....	20:100 or 1:5
1.010 - 1.020 .....	15:100 or about 1:6½
1.020 or above .....	10:100 or 1:10

One c.c. of the diluted urine is pipetted into a Pyrex nitrogen tube, and 2 c.c. of acid digestion mixture (see section on Blood, footnote No. 4) added. A glass bead is put into the mixture to prevent bumping. The mixture is now digested over the microburner, the same as in the determination of non-protein nitrogen constituents in the blood. After the addition of water the solution is transferred to a 200 c.c. volumetric flask. In another 200 c.c. flask a standard is made up containing 5 c.c. of the ammonium sulphate standard solution, 2 c.c. of acid digestion mixture, and about 125 c.c. of water. To each are added 30 c.c. of Nessler's solution (see section on Blood, footnote No. 6.) and water to the mark. Readings are made against the standard and calculated according to the formula:

$$\frac{\text{Standard}}{\text{Reading}} \times \text{dilution} \times \frac{1}{1000} \times 24 \text{ hour volume} = \text{gm. of total nitrogen in 24 hours.}$$

**UREA NITROGEN.**—Urea nitrogen varies normally between 3 to 5.5 grams in 24 hours, or about 80 per cent of the total nitrogen. The amount is decreased in nephritis with retention and in uremia. It also varies in small limits with the amount of protein ingested.

**Method of Determination.**—One c.c. of the diluted urine (see total nitrogen in urine) is measured into a Pyrex tube. One drop of buffer mixture (see section on Blood, footnote No. 7), and one c.c. of urease solution (footnote No. 8) are added. The mixture is placed in water at 55° C. and allowed to stand for ten minutes. It is then transferred to a 200 c.c. volumetric flask. The standard is now prepared, consisting of ammonium sulphate standard solution, 1 c.c. of urease solution, and about 100 c.c. of water. Twenty c.c. of Nessler's solution are added to each, and water to the mark. Readings are made against the standard and the result calculated according to the formula:



$$\frac{\text{Standard Reading}}{\text{Reading}} \times \text{dilution} \times \frac{1}{1000} \times 24 \text{ hour volume} = \text{gm. of urea nitrogen in 24 hours.}$$

A less accurate method of determining urea in the 24 hour quantity of urine, but one used extensively by clinicians is the hypobromide method, which consists of filling the Doremus' ureometer with sodium hypobromide and instilling 1 c.c. of urine into the ureometer with a curved pipette. The gas replacing the solution on the top is read off by the number on the ureometer and converted to percentage. Thus a reading of 0.01 equals 1 per cent urea, etc.

**CREATININE.**—The amount of creatinine in children's urine varies between 0.3 to 0.5 gm. in 24 hours.

**Technic.**—Into a 100 c.c. volumetric flask is measured 1-3 c.c. of urine. Into a similar flask is measured 1 c.c. of standard creatinine solution containing 1 mg. of creatinine (see creatinine in blood). To each is added 20 c.c. of saturated picric acid solution, and then, noting the time, 1.5 c.c. of 10 per cent NaOH. After standing for just ten minutes, the solutions are made up to the mark with water and read in the colorimeter, the standard being placed at 20.

$$\frac{\text{Standard Reading}}{\text{Reading}} = \text{mg. of creatinine in volume of urine taken.}$$

**CHLORIDES.**—The amount of chlorides in normal urine varies between 2 to 4 gm. per 24 hours or between 0.7 to 1.0 per cent. Anything below 0.7 per cent speaks for the possibility of a threatening edema. The quantitative determination of chlorides therefore becomes a valuable kidney function test.

Two solutions are prepared for the test. Solution 1 consists of the following:

Anhydrous, crystallized silver nitrate, (C. P.) .....	29.055 gm.
25% Nitric acid in distilled water .....	900. c.c.
Cold saturated solution of ammonioferrie alum in distilled water ....	50. c.c.
Distilled water, q. s. ....	1000 c.c.

Solution 2 consists of:

Ammonium sulphocyanate .....	7 gm.
Distilled water, q. s. ....	1000 c.c.

Solution 2 is intentionally made too strong, and must be standardized by adding distilled water in such an amount that exactly the last drop of 2 c.c. of this solution will bring about the end reaction when added to 1 c.c. of Solution 1. The end reaction consists of a reddish brown color, which does not disappear on moderate stirring. If the second last drop produces a discoloration which disappears rather slowly, and the last drop a deep brown color, the solution must be still further diluted, until the discoloration on the addition of the last drop is a light reddish-brown, which does not disappear on stirring fifteen to twenty seconds.

The test proper is made as follows: 0.5 c.c. of urine to be tested is placed in a porcelain dish, 1 c.c. of Solution 1 is then added and the mixture is stirred for about a minute with a glass rod. Solution 2 is now added drop

by drop by means of a 2 c.c. pipette graduated to at least .05 c.c. and the mixture is stirred until the brown color developing after each drop disappears. The amount of Solution 2 which has been used to bring about the end reaction is now read off, and the difference between this and 2 is equal

TABLE X

## CONSTITUENTS OF URINE IN NORMAL CHILDREN

Amount in 24 hours	360 to 480 c.c.
Specific gravity	1010 to 1018
Albumin	Negative
Sugar	"
Acetone	"
Casts	"
Cells	Few
Chlorides	0.7-1.0%
Chlorides (in 24 hours)	2 to 4 gm.
Total Nitrogen (in 24 hours)	4.0 to 7.2 gm.
Urea Nitrogen (in 24 hours)	3 to 5.5 gm. (80% of total nitrogen)
Creatinine (in 24 hours)	0.3 to 0.5 gm.
Ammonia Nitrogen	0.7 to 1.2%

to the number of grams of sodium chloride per 100 c.c. of the specimen tested. If, for example, it takes 1.26 c.c. of Solution 2 to bring about the end reaction, the amount of chloride in 100 c.c. of urine equals  $2 \times 1.26$  which equals 0.74 per cent of chlorides.

TABLE XI

CHEMICAL CONSTITUENTS OF BLOOD AND URINE IN SAME CHILD (NORMAL)  
BLOOD

Mg. per 100 c.c.

Nonprotein Nitrogen	Urea Nitrogen	Uric Acid	Creatinine	Chlorides	Sugar
26.1	12.6	3.2	1.1	5.75	100

## URINE

c.c. per 24 hours

Quantity in 24 hours	Total Nitrogen	Urea Nitrogen	Creatine	Creatinine	Chlorides
540	4.56	4.10	0.037	0.34	3.93

KIDNEY FUNCTION TESTS.—The two most commonly used tests are: The phenolsulphonephthalein and the Mosenthal.

PHENOLSULPHONEPHTHALEIN TEST.—A sterile solution of phenolsulphonephthalein, containing 6 mg. of the dye per 1 c.c. (may be obtained in ampoules) is injected subcutaneously. The patient is given all the water he wants during the test so as to cause diuresis. Ordinarily, the dye will appear in the urine in 10 minutes. Urine is collected during the first, second, and third hour in separate bottles.

Each specimen of the collected urine is made alkaline with 25 per cent sodium hydrate, and diluted to 1 liter, or less, according to the amount of urine, and the readings are made by comparison with standard color tubes obtained on the market, or by means of a Hellige colorimeter. Blood, if

present, may be removed by using powdered lime or milk of lime, instead of sodium hydrate. This makes the solution alkaline and precipitates the blood pigments.

In normal children 70 per cent to 80 per cent of the injected phenol-sulphonephthalein is excreted in 2 hours. Forty to 50 per cent the first hour, and 20 to 30 per cent the second hour. In nephritis, the excretion of the dye is usually lowered according to the damage to the kidneys.

The phenolsulphonephthalein test, I believe, is only corroborative, but no diagnosis or prognosis should be based on it. I have seen patients in whom the phenolsulphonephthalein excretion was down to 5 per cent, who recovered, and others with 80 per cent phenolsulphonephthalein excretion, who died of insufficient kidney function.

**MOSENTHAL TEST.**—The test lasts 24 hours. The bladder is emptied at 8 A. M. and the urine discarded. The patient is given his customary meal, and in addition, 1 quart of fluid. The same is done at noon, and at 5 P. M. No food is allowed at any other time. The amount of fluid may be increased or decreased according to the weather and to the age of the child.

The urine is collected in 2 hour specimens until 8 P.M., the patient voiding at the end of each 2 hour period in order to make each specimen complete. The night urine, from 8 P.M. to 8 A.M., is collected as one specimen.

The volume and specific gravity of each specimen is measured. When there is a disturbance in kidney function, the quantity of night urine increases above normal and shows a low specific gravity. In advanced functional disturbances of the kidneys, the specific gravity becomes fixed and low.

## REPORT OF A CASE OF ERYTHREMIA\*

BY IRWIN S. SUTTON, M.D., AND WILLIAM COLE, M.D., ANAHEIM, CALIF.

CASE No. 12753.—Mr. M. S. W., a widower, aged 49, complained of soreness and swelling of the left knee.

Family History.—His father died at 50 of a tropical fever and his mother at 51 from tuberculosis. He has three brothers all of whom are healthy adults. His wife died, aged 51, of consumption and had never been pregnant.

Past History.—He had never had malaria, tuberculosis, or syphilis, but in 1914 had had a severe attack of typhoid fever. The same year he received a severe blow to the left patella which he believes was fractured. After rest in bed for several months he made a complete recovery. While in the hospital in 1921 because of a crushing injury of the left foot he developed a collection of fluid in the scrotal sac without apparent reason, and which disappeared without any treatment. Lately he has noted moderately severe temporal throbbing headaches which are gradually increasing in intensity.

Present History.—In November, 1922, he suddenly developed a large amount of fluid about the left knee joint which was tender on motion. The swelling increased under the ministrations of a chiropractor and has lately interfered with sleep. Recently he has had several attacks of epistaxis but no subcutaneous hemorrhages. There have been a few fleeting joint pains and some embarrassment of the heart on exertion. He has lived for twenty years in this locality where the altitude is low. Since October, 1922, he has had nocturnal and diurnal frequency of urination. Several of his friends have recently complimented him upon his ruddy complexion.

Physical examination.—The patient was a middle-aged, poorly nourished, stoop shouldered man, markedly erythrotic. The conjunctivæ were extremely injected, and the face, neck and hands were of a dark reddish hue, changing to a dusky purple on exposure to a draught of cold air. The tongue and oral mucosa were of an intense scarlet tint. The hair and nails were rather dry and lusterless and there was a pronounced tendency to ichthyosis. Pupils were concentric, equal and active. Ocular movements were normal. Ophthalmoscopic examination revealed engorgement of the retinal vessels and moderate edema of the nerve heads.

The nasal examination.—The nasal mucosa and turbinates were congested. There was a superficial ulceration of the septum on the left. The gums were spongy and bled easily on pressure. Roentgenograms revealed two infected teeth. There was a slight general adenopathy. There was no demonstrable thyroid enlargement.

Chest examination.—There was some flattening of the left side anteriorly and a marked depression at the xiphoid. There was also some general

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From the Johnston-Wickett Clinic, Anaheim, Calif.



emaciation. The breath sounds were rather harsh throughout and some crackles were heard over the splenic area which suggested a perisplenitis. Stereo-roentgenograms revealed a normal heart shadow and a few fibrous bands in the upper part of both lungs. The cardiac examination was essentially negative but the blood pressure was rather high (systolic 170, diastolic 110).

Abdominal examination.—The skin was rather pale, contrasting with the general erythrocytic appearance. The abdomen was rather tympanitic and prominent below the umbilicus. Liver dullness extended from the fifth rib in the nipple line to the costal margin. It was impossible to palpate the spleen. The external genitalia were negative and the prostate was not enlarged.

Orthopedic examination.—The general carriage was slouchy, with some kyphos and bowing of the legs. The left knee joint was tense from a collection of synovial fluid. There was no muscle spasm, local heat, or redness. A roentgenogram showed a slight roughening of the articular surfaces. Aspirated fluid was sterile. With rest in bed and a pressure bandage the fluid rapidly disappeared. There was tibial periostitis. Neurologic examination was negative except for a weak to absent right ankle jerk.

A twenty-four hour urine specimen contained a strong trace of albumin, specific gravity was normal and the specimen was highly acid. The serum Wassermann reaction (two antigens) negative. Basal metabolism, average of the readings was +42.7. A glucose tolerance test showed a normal curve.

Blood examination.—Blood sugar 0.17, creatinine 1.8 mg., uric acid 2.7 mg., nonprotein nitrogen 52.4 mg., chlorides 0.6 per cent.  $\text{CO}_2$  tension (Van Slyke) 68.3 volumes per cent. Red cell fragility test—Hemolysis began at 0.48 per cent and was complete at 0.28 per cent. The clotting time varied from two to four minutes and stained and unstained blood smears showed normal erythrocytes.

The hemoglobin (Dare) was estimated at 202 per cent and erythrocyte count was 10,760,000. This was checked with a U. S. certified pipette and found accurate. The color index was 0.9445. The leucocyte count was 9,200, with 76 per cent polynuclears; 19 per cent small mononuclears, 3 per cent large mononuclears and 2 per cent transitional cells.

Discussion.—This patient complained of a painful swollen knee and was unaware of any blood changes. His color suggested heart disease but this organ was practically normal. The low blood sugar and the negative urine eliminated diabetes as a cause of the polycythemia. The fact that his wife and mother died of pulmonary disease would make it rather difficult to eliminate this factor. He has lived for some time at a mean altitude of thirty feet above sea level and has not had malaria. The joint lesion disappeared under pressure bandages and did not at any time suggest tuberculosis. The fluid accumulation was possibly due to a change in blood volume, associated with some trauma of the knee. A review of the literature shows the blood count in this case remarkably high and that it is the only case reported with joint symptoms.

# A COMPARATIVE STUDY OF VARIOUS METHODS OF HEMOGLOBIN DETERMINATIONS\*

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## INTRODUCTION

HEMOGLOBIN is the oxygen-carrying constituent of the blood. It is a complex proteid, which, on decomposition, breaks up into globulin (96 per cent) and a simpler pigment, hematin (4 per cent<sup>10</sup>). The iron content is about 0.334 per cent, or 1 mg. for each 300 mg. of hemoglobin. The estimation of hemoglobin is a very important procedure in clinical diagnosis; it is of value in determining whether patients with pallor are really anemic. Then, too, the hemoglobin content of the blood is a necessary factor in determination of the color index in diseases such as pernicious anemia and chlorosis. The normal hemoglobin in adults is 13 to 14 gm. for each 100 c.c. of blood (100 per cent). The hemoglobin in infants during the first few days of life is very high, usually about 130 to 135 per cent; in young children 75 to 80 per cent; in adults 95 to 100 per cent, while the percentage is usually lower than 100 per cent after the age of sixty-five years. Haldane and Smith assert that the depth of color in the blood is directly proportional to the oxygen capacity, the percentage of hemoglobin present in other words. In the estimation of hemoglobin, various methods have been employed, aiming at accuracy and ease of determination.

## HISTORY OF THE DEVELOPMENT OF EXISTING METHODS

In 1878, Gowers introduced his hemoglobinometer. This instrument consists of two tubes about 11 by 0.8 cm. One tube, closed at both ends, contains the standard solution, 2 c.c. of gelatine tinted with picrocarmin. The color of this standard corresponds as nearly as possible to a 1 per cent hemoglobin solution. In the other tube the level attained by 2 c.c. of fluid will represent 100 per cent of hemoglobin. This tube is graduated to read hemoglobin percentage from 10 to 140 per cent. In making a hemoglobin estimation, 0.20 c.c. of blood is obtained in a graduated pipette and blown into the graduated tube which contains a little water. This is thoroughly mixed and the pipette washed by frequently drawing the solution into the pipette. Water is added, drop by drop, and the solution intimately mixed, until the colors in both tubes are similar. The percentage of hemoglobin is then read at the height of the solution in the graduated tube. This instrument is very simple, handy and inexpensive, but only fairly accurate; the picrocarmin solution fades and

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the same standard tube cannot be used with artificial light. Then, too, the standard solution is not made of blood and consequently there is some difficulty in the accurate matching of the solutions.

Fleischl,<sup>16</sup> in 1885, devised a new hemoglobinometer which has since been much improved by Miescher, now known as the Fleischl-Miescher instrument, consisting of a stage similar to that of a microscope. In the center is placed a cylindrical chamber divided into two equal parts and having a glass bottom. Half of the chamber is filled with water, the other half with a 1:200 dilution of blood with a 1 per cent solution of sodium carbonate. A purple-stained, glass wedge, or color prism, with the hemoglobin percentage values marked along the edge, slides beneath the chamber, and is moved from side to side by a screw. Light from a candle is reflected by a plaster-of-Paris reflector and illuminates the chamber from below. The glass wedge beneath the chamber is moved back and forth until the color of the portion of the wedge beneath the chamber containing the water is similar to that of the blood solution in the opposite chamber. The position of the wedge is read through a small window at the back of the stage, and represents the percentage of hemoglobin present. Each instrument has a scale which gives the milligrams of hemoglobin for each liter of blood according to the readings made by that particular hemoglobinometer. When the dilution of blood is known, it is possible to determine the amount of hemoglobin in grams for each 100 c.c. On account of the accuracy of this instrument, it has always been popular in experimental work. It is, however, quite cumbersome and expensive, and must be used in a dark room with a yellow light; these objections, together with the time required and the technical difficulties make it impractical for general clinical use.

In the year 1892, Hoppe-Seyler described a method of determining the percentage of hemoglobin, whereby the blood to be examined was saturated with carbon monoxide. This solution was compared with a known carbon monoxide standard solution. The method has proved too complicated for general clinical use.

Haldane and Smith, in 1898, suggested a new method of estimating the hemoglobin content of the blood. They noticed that, on the addition of potassium ferrieyanide to a moderately diluted solution of blood, gas bubbles arose which were found to be pure oxygen. With this in mind, they devised an instrument for liberating and measuring the oxygen from the blood, and determined the oxygen capacity of normal blood of the adult male to be 18.5 volumes per cent, which was taken as an equivalent of 100 per cent hemoglobin; that of women 16.5 volumes per cent, and that of children 16.1 volumes per cent. The method has been much improved technically by Haldane, Bancroft, Van Slyke and others. While not practical for routine hemoglobin determination, it is nevertheless, one of the most accurate methods. It is of inestimable value in the preparation of standard hemoglobin solutions.

Tallqvist's simple and unique hemoglobinometer was introduced in 1900. This instrument consists of a booklet of prepared white filter paper and a lithographed color scale representing the hemoglobin content of blood to be

from 10 to 100 per cent. The tints of the standard are prepared with water colors, and represent the color produced by undiluted blood soaked in filter paper, the hemoglobin values having been determined by the Fleischl-Miescher instrument. A rather large drop of blood is touched to a piece of the filter paper and allowed to distribute itself over a portion of it. As soon as the stain has lost its humid gloss, and before coagulation can take place, the stained filter paper is laid beneath the circular openings of the color scale, and at the point where the colors match, the percentage of hemoglobin is read. Because the colors of the scale vary by 10 per cent, an approximate value only can be obtained, and this reading may vary if the color is matched too soon or after coagulation has begun. The method cannot be recommended for accuracy in routine clinical use.

In 1900, also, Dare devised a new instrument for hemoglobin estimation. The use of this instrument is based on the premise that the color of a thin film of undiluted blood illuminated by artificial light can be compared with

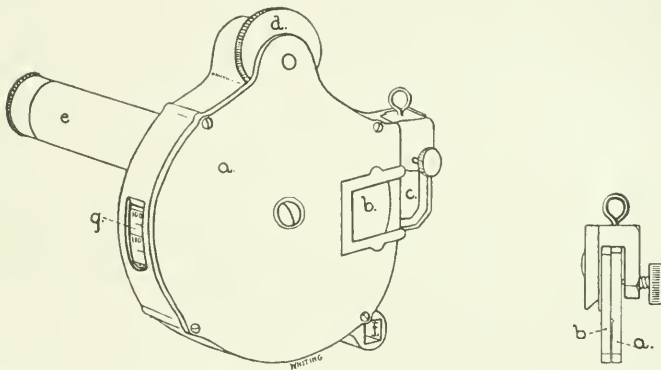


Fig. 1.—Dare hemoglobinometer and capillary pipette of Dare hemoglobinometer.

a graduated color comparison standard. This standard is a color prism made of ruby glass; the 100 per cent reading represents 13.7 gm. of hemoglobin for each 100 c.c. of blood. Hemoglobin estimations can be made from 10 to 120 per cent.

The instrument (Fig. 1) consists of a case (a) enclosing the color-prism comparison standard, with an aperture (b) in the case, admitting light to the color prism, a pipette (c) made of two thin pieces of glass, one opaque and one transparent, with a surface of definite thickness (about 0.18 mm.) between; a small screw (d) to move the color prism; telescope (e) to focus and magnify; an opening (f) for the attachment of the candle or the electric light equipment, which is part of the newer instrument. For the purpose of color comparison, blood is drawn by capillary attraction into the small thin chamber of the pipette which has a sufficient surface for color field and illumination by artificial light. Between the film of blood and the source of illumination, a white glass interposes to diffuse the direct rays of light. The shades of color are best observed against this white background. The pipette is slipped into the instrument (Fig. 1b), candle or artificial light is



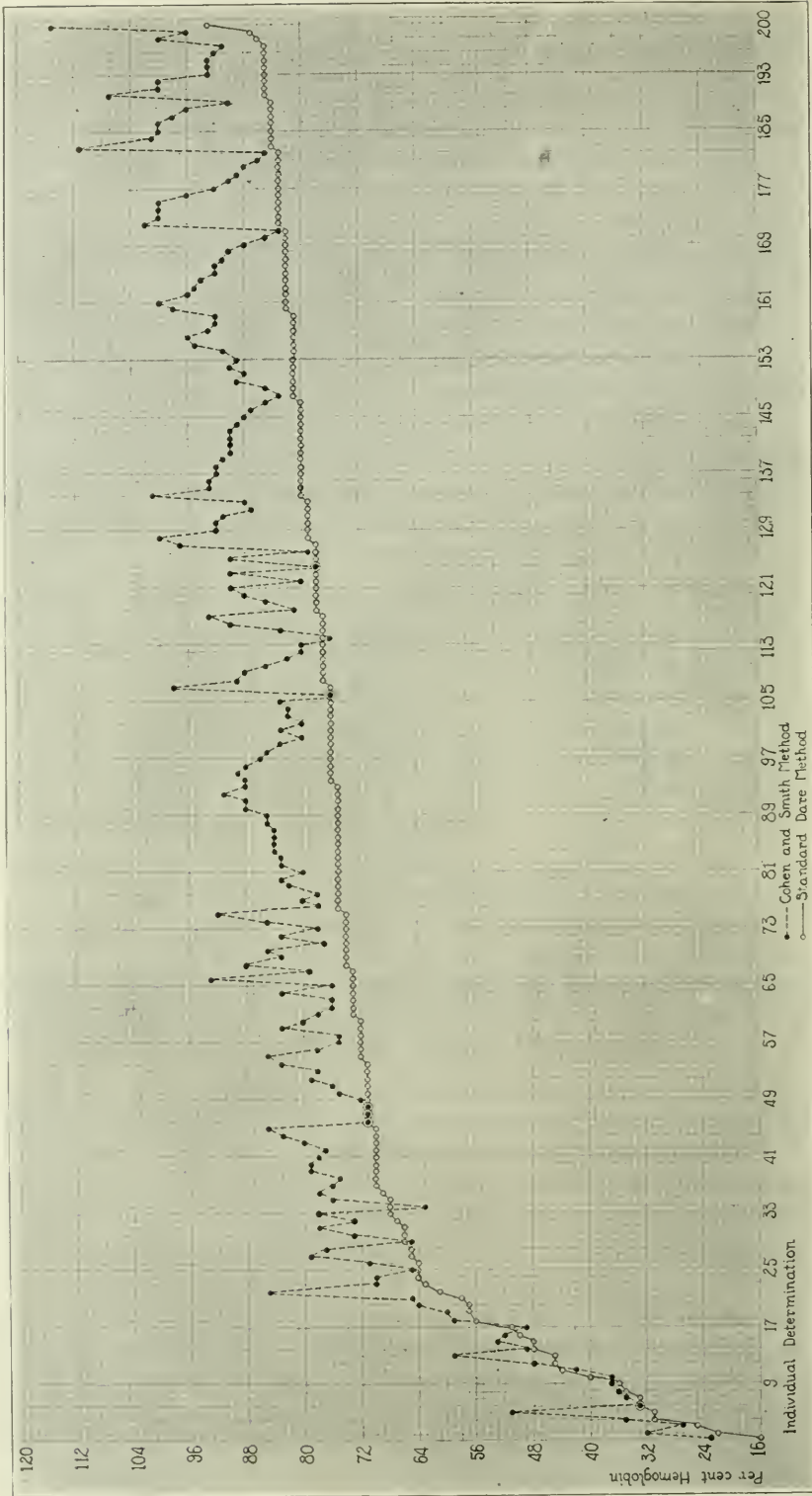


Fig. 2.—Standard Dare readings and readings by the Cohen and Smith acid hematin method. Except in isolated cases, readings by both methods check up to approximately 65 per cent. Above 65 per cent, and especially above 70 per cent, the calculations on the standard Dare as compared with those obtained by the Cohen and Smith method, show marked variations.

used, and the prism is rotated until the colors match, when the hemoglobin percentage is read through the notch (g) at the edge of the case. As suggested by Dare the advantages of his instrument are:

"1. The technic and dilution errors are eliminated. Turbidity due to leukocytosis becomes almost imperceptible with transmitted light against an opaque background.

"2. The shades of color are very decided even though the hemoglobin content is low.

"3. The time element is very short.

"4. The capillary pipettes are standardized and are consequently interchangeable."

This instrument is very simple and convenient, but quite expensive and must be handled carefully.

Haldane described a revised method of hemoglobin determination in 1901. He used the Hoppe-Seyler principle with the Gowers' hemoglobinometer and claimed very satisfactory results. The standard solution used is a 1 per cent solution of ox or sheep's blood saturated with carbon monoxide and having an oxygen capacity of 18.5 volumes per cent, which value has been quite generally accepted and represents 100 per cent hemoglobin. In using this method 20 c.c. of blood is delivered to the graduated tube, containing as much water as safely possible. Before mixing, a narrow glass tube, connected with a gas tap, is pushed down almost to the level of the liquid. The gas is turned on, displacing the air in the tube. The glass tube is then slowly withdrawn, with the gas still flowing, and the top of the graduated tube quickly closed with the finger. The solution is thoroughly mixed until the pink tint of carbon monoxide hemoglobin appears. Water is added, drop by drop, from a pipette until the tints in both tubes are similar. The percentage is then read at the height of the fluid in the graduated tube. Another drop or two is added to produce a change in color, and the mean of the readings is accepted as the correct percentage of hemoglobin present. The tints are best compared against sunlight or an opal glass light if artificial light is desired. This method is fairly accurate but, as asserted by Palmer, the standard is not permanent.

Perhaps the most simple and popular hemoglobinometer in use today is that described in 1902 by Sahli who, for the first time, used an acid hematin standard. The instrument is similar to that of Gowers except that the standard is a 1 per cent solution of acid hematin instead of a picrocarmin solution corresponding to a 1 per cent solution of blood. The blood to be tested is likewise changed into acid hematin. In determining the percentage of hemoglobin 0.20 c.c. of blood is obtained in the Sahli pipette and transferred to the graduated tube which contains 0.1 normal hydrochloric acid up to 10 per cent. The tube is well shaken, and when the clear, dark brown color due to the formation of acid hematin, appears, distilled water is slowly added and the contents carefully and thoroughly shaken until the color of the mixture corresponds to that of the standard solution. At this point the percentage of hemoglobin is read: it corresponds to the level of the solution in the grad-

uated tube. This instrument has a ground glass background which diffuses the light before it reaches the tube, thereby excluding all disturbing reflections. It can be used with artificial light and in a lighted room. The color comparisons, of course, will be more accurate if the standard and tested solutions are of similar composition. For this reason the Sahli instrument is superior to that of Gowers or to other methods using foreign standard solu-

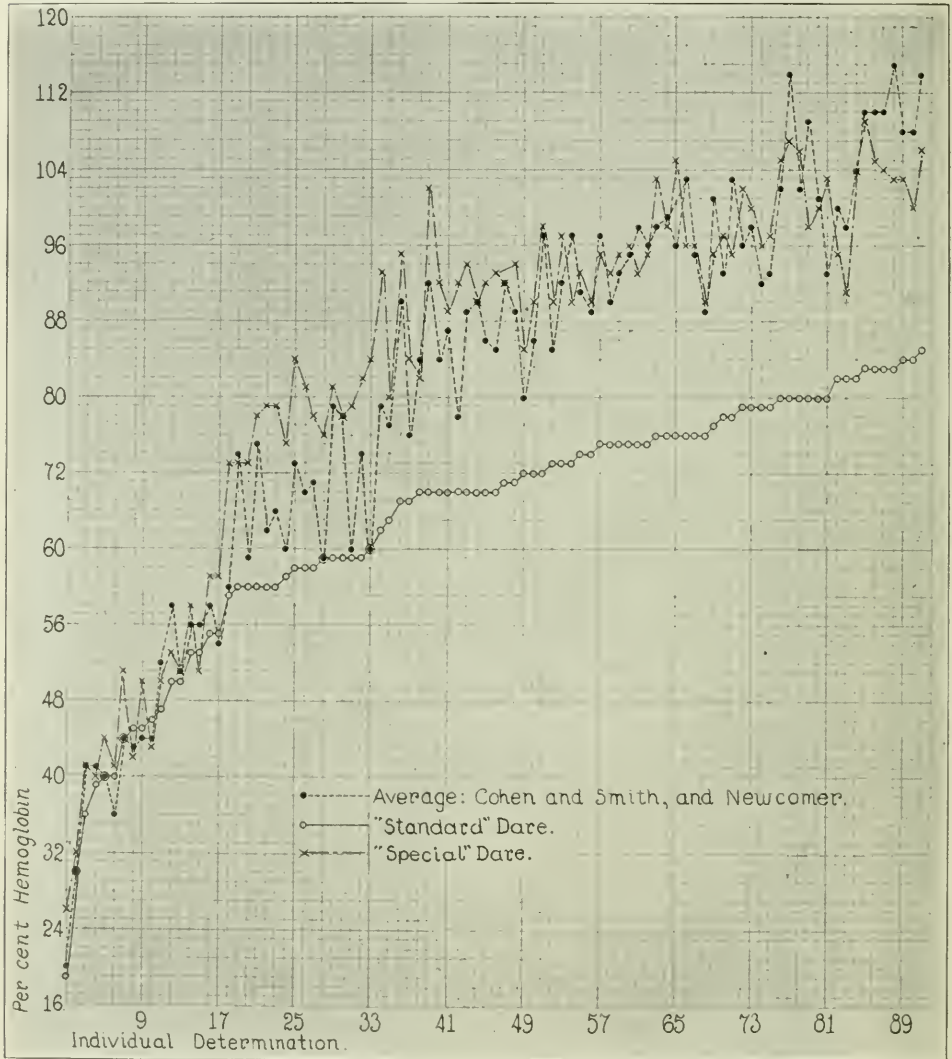


Fig. 3.—Average readings by the Newcomer and Cohen and Smith methods and readings by the standard and special Dare instruments. In this curve also, the values are similar up to approximately 60 or 65 per cent. Readings of from 60 to 80 per cent on the standard Dare follow as in Fig. 2, while the special Dare reads approximately 10 to 15 per cent higher. The readings on the special Dare, in determinations from 80 to 115 per cent, compare favorably with the average readings by the Cohen and Smith, and Newcomer methods.

tion or colored glass as standards. Sahli tubes on the market today fade so markedly as to make them unfit for use, other than comparison, unless frequently checked and restandardized. In the Sahli method of hemoglobin estimation 17.2 gm. for each 100 e.c. represents 100 per cent, and the deter-



minations must consequently be reduced if 13.4 gm. for each 100 c.c. is to be considered as standard. Robscheit says that the tubes purchased today show fading varying from 5 to 20 per cent. Jacobson has suggested a new standard for the Sahli hemoglobinometer, "rufigallic acid." He makes this solution by adding 5 to 10 drops of concentrated sulphuric acid to 100 c.c. of a

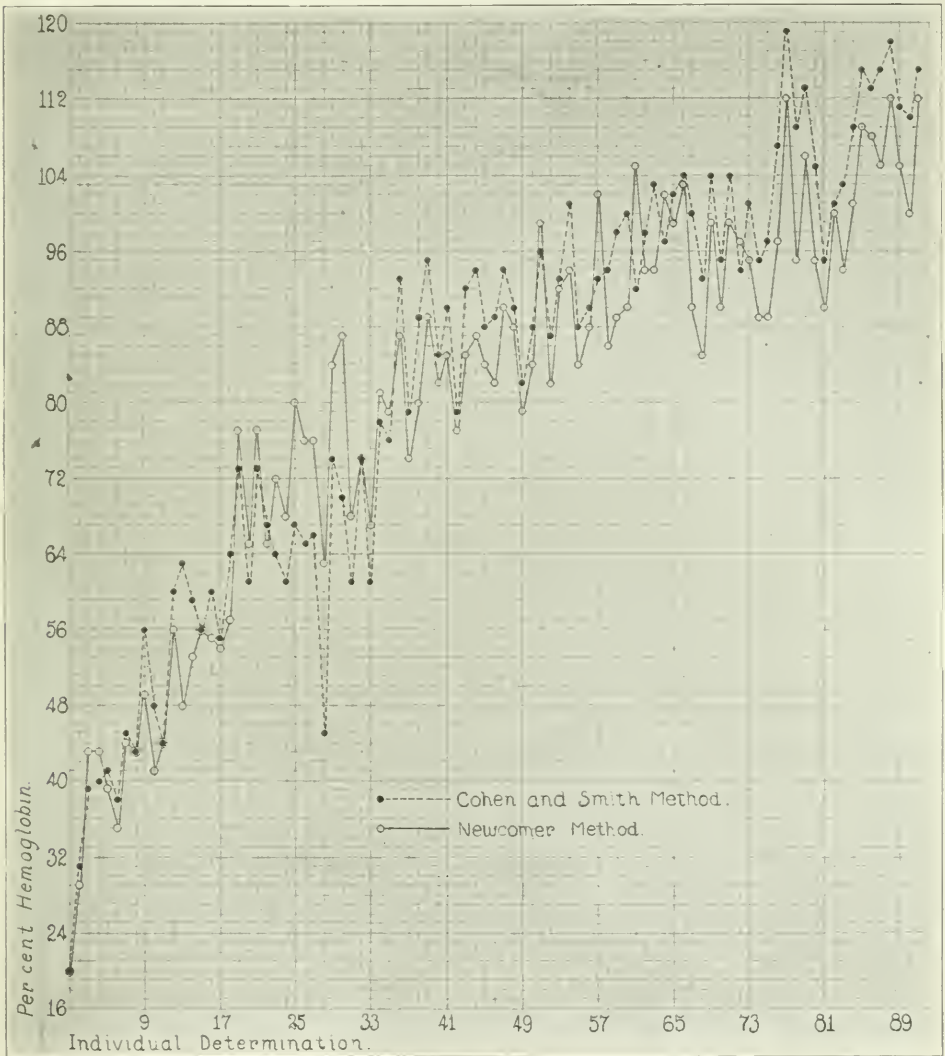


Fig. 4.—The marked uniformity between the Newcomer, and Cohen and Smith acid hematin methods is demonstrated. With but few exceptions, the variations are within the limits of error. From the clinical point of view these methods are equally accurate and interchangeable.

20 per cent aqueous solution of tannic acid, or a 1 per cent solution of gallic acid. After heating this for one minute, a dark brown mixture results. The dilution desired can then be made, and corresponds very closely to an acid hematin solution when viewed in a Duboseq colorimeter. Jacobson claims



that a 20 per cent solution has remained in the sunlight for ten months with no apparent change of color.

In 1916 Haessler and Newcomer devised another acid hematin hemoglobinometer adopting the principle of Sahli's instrument, but using eleven standard tubes arranged in a rack instead of but one. The readings on these tubes vary by 10, and represent hemoglobin readings from 10 to 110 per cent. The standard fluids are made up according to Sahli's specifications. The tube marked 100 per cent contains an equivalent in acid hematin of 17.2 gm. of

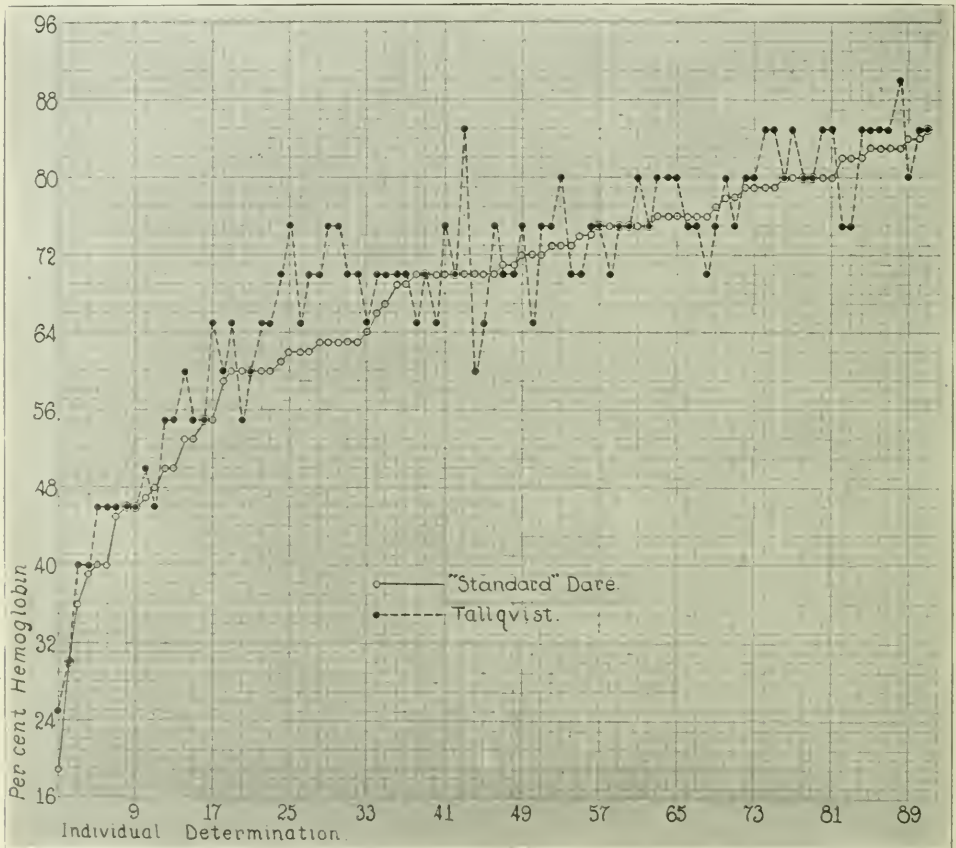


Fig. 5.—The striking similarity is shown between hemoglobin estimations made on the Tallqvist and Dare hemoglobinometers. This curve suggests strongly that if directions are followed with care, the Tallqvist determinations will be almost as accurate as those obtained on the standard Dare instrument.

hemoglobin in 10,000 c.c. The patient's blood is obtained in an ordinary, red blood pipette, a 1:100 dilution is made with 0.1 normal hydrochloric acid, and the solution compared with the standard tubes in the rack. The tube containing the blood to be tested, or the comparison tube, is inserted between the different tubes of the standard and moved one way or the other on the rack until the shading becomes harmonious, or as nearly so as possible. The per cent of hemoglobin is then read or estimated. As the readings on the

instrument vary by 10 per cent, the values are obviously only approximate. For this reason the percentages obtained are of value only as a comparison.

The method of determining hemoglobin described by Palmer, in 1918, is rapidly becoming popular, and is recognized as a standard method today. It is a comparison, by means of a colorimeter, of carbon monoxide hemoglobin solutions, one solution having a known hemoglobin content. The principle is similar to that described by Hloppe-Seyler in 1892. The standard solution is prepared as follows:

A quantity of ox or human blood is defibrinated. The oxygen capacity is determined according to the method of Van Slyke. The blood is then diluted with 0.4 per cent ammonium hydroxide solution so as to make a 20 per cent solution having an oxygen capacity of 18.5 volumes per cent. This solution is saturated with carbon monoxide by bubbling ordinary illuminating gas through it for ten minutes. It is immediately plugged, preferably with a glass stopper. This standard, 20 per cent solution should then be sealed and kept in the dark on ice. When used for hemoglobin estimation, a 1 per cent solution is made. Five cubic centimeters of this 20 per cent solution is diluted to 100 c.c. with 0.4 per cent ammonium hydroxide solution and saturated with carbon monoxide. A 1 per cent solution of a patient's blood is made by transferring 0.05 c.c. of blood into 5 c.c. of 0.4 per cent ammonium hydroxide solution. The blood pipette is rinsed by drawing the ammonia solution into it three or four times. Illuminating gas is then bubbled through the solution for thirty seconds. This solution is compared in a Duboseq colorimeter with the standard 1 per cent solution set at 10. The average of several readings is taken. The calculation is  $\frac{10}{R} \times 100 =$  per cent of hemoglobin. The color match is excellent.

Considerable difficulty is met with in obtaining standards which will remain constant. Robschelt records changes in color value of Palmer standards of 10 to 20 per cent in a period of from seven to twelve months. If standards are prepared once a month, however, very accurate readings are obtained.<sup>15</sup> It is not always possible to obtain illuminating gas, which adds further difficulties.

In 1919, Newcomer presented his method of estimating hemoglobin by the use of a carefully prepared "high transmission yellow" semaphore glass disk. This instrument is based on the spectro-photometric properties of acid hematin. These thin glass disks of known thickness (about 1 mm.) and known hemoglobin values are used in place of the standard solution in the colorimeter. The disk is placed in the light path of one of the cups of the colorimeter (at the top of the plunger) and the corresponding cup is filled with water. In the other cup is poured the solution to be read. This consists of blood diluted with 0.1 hydrochloric acid. Five c.c. of 0.1 normal hydrochloric acid is measured with a pipette into the colorimeter cup or a small tube. Twenty c.mm. of blood is emptied and rinsed into this solution. The height of the color, produced by the acid hematin formed, appears in about forty minutes. The colors are then matched and the reading on the colorimeter is noted. A

scale accompanies the disk, and the hemoglobin percentage or the grams for each 100 c.c. is obtained by dividing the reading on the colorimeter into the appropriate figure in the scale. This scale is corrected both for time factor before matching, and for the thickness of the standard disk used. The estimations of hemoglobin are so satisfactory with this instrument that these disks are now made a part of the regular equipment of the Bausch and Lomb colorimeter of the Duboseq type. The new Bock and Benedict colorimeter is also well adapted for use with the Newcomer disk.

In 1919, Cohen and Smith described a practical method of accurately determining hemoglobin. Like Palmer, they use a colorimeter, but employ an acid hematin standard. The authors say that this method was devised for field use and has proved very satisfactory in the United States Army.

*Preparation of Standard Solution.*—About 50 c.c. of blood (ox or human) is defibrinated. The oxygen capacity is determined by the Van Slyke method. The blood is then diluted with 0.1 normal hydrochloric acid to make a 20 per cent stock solution with an oxygen capacity of 18.5 per cent. Such a standard will contain approximately 14 gm. of hemoglobin for each 100 c.c. This solution is treated with a few drops of chloroform to prevent mold formation, and is then placed on ice in a dark, glass-stoppered bottle. The comparison standard to be used in the colorimeter is a 0.5 per cent solution, prepared by diluting 2.5 c.c. of stock standard solution with 0.1 normal hydrochloride acid up to 100 c.c. By means of a calibrated Sahli pipette 0.02 c.c. of blood is obtained and added to 6 c.c. of 0.1 normal hydrochloride solution. The pipette is carefully washed several times by drawing the solution into it. The solution is then poured into one of the cups of the colorimeter and compared with the 0.5 per cent comparison standard. The average of several readings is accepted as correct.

With the standard solution a 0.5 per cent blood solution, and the Duboseq colorimeter set at 10, the calculation will be as follows:

$$\frac{1.5 \times 10 \times 100}{R} = \text{the percentage of hemoglobin.}$$

Cohen and Smith assert that the stock standard solution will remain stable at least three months. They find that accurate results are obtained by comparatively unskilled workers. The results are very accurate and the color match is good. Dilution errors must be carefully eliminated.

A method similar to that of Newcomer was described in the literature by Dreyer, Bazett and Pierce, in 1920. Dreyer says that for ten years he has used a hemolyzed solution of blood and compared it in a Duboseq colorimeter, using a colored glass as a standard. In making such determinations a 1:100 dilution of blood is made by diluting 0.1 c.c. of blood with 19.9 c.c. of normal saline. This solution is intimately mixed, and may be placed on ice until convenient to make the reading when it is hemolyzed by using a very minute amount of saponin. This can be done in a warm room by gently rotating the solution, but preferably by heating for thirty minutes in a water bath at about 30° C. The hemolyzed solution is then poured into one of the

cups of the colorimeter and matched against the pink glass standard. Artificial light is used, preferably one rich in yellow rays. The results are more constant if the readings are made in a dark room and when the reflector only is illuminated. The authors emphasize the variations in hemoglobin readings taken at different hours of the day, and regard the variations to be the smallest from 5:00 to 7:00 P.M.

The hemoglobin estimations made by the Cohen and Smith, Palmer, Newcomer and Dreyer methods, using a Dubosecq colorimeter, are similar and very accurate.

#### A COMPARISON OF VARIOUS METHODS OF HEMOGLOBIN DETERMINATION

The Dare instrument is used at the Mayo Clinic. Because the determinations were so low in apparently normal individuals, it was thought advisable to check the method as well as to ascertain the value of other methods

TABLE I

PALMER'S METHOD	NEWCOMER'S METHOD (GLASS 0.96 MM. THICK)	DIFFERENCE
<i>per cent</i>	<i>per cent</i>	
113	114	+1
116	114	-2
99	100	+1
107	108	+1
98	101	+3
117	118	+1
100	100	0
100	100	0
100	101	+1
100	103	+3
100	99.2	-0.8
Average difference.....		+0.73

for general use. No satisfactory investigations relative to the value of the Dare hemoglobinometer were found in the literature.

In this experimental work the Newcomer disk, the acid hematin method as described by Cohen and Smith, the Tallqvist hemoglobinometer, and two Dare instruments, a "standard" Dare hemoglobinometer and a "special"\* Dare hemoglobinometer were used.

Patients of the Clinic sent to the hematology laboratory for hemoglobin determination and healthy Clinic workers were examined exclusively in this investigation.

*Newcomer Disk Method.*—The study by this method proved very satisfactory. The procedure is simple and the determination can be accomplished quickly. One disk, 1.0 mm. in thickness, was used. There was some difficulty at times in matching colors, especially when the hemoglobin readings were low. Light was supplied by a northern exposure and an electric light with Corning "daylight glass." The electric light with the daylight glass, being always constant, was thought superior to daylight. In Robscheit's

\*Because of the findings in Fig. I, Dr. A. H. Sanford, of the Mayo Clinic, had a "special" Dare hemoglobinometer made by the Ricker Instrument Company, of Philadelphia, in which the glass prism comparison standard was ground 15 per cent thinner at the upper end of the scale than that of the "standard" Dare.



work it is noticed that the Palmer method was compared with the Newcomer disk, and that the readings were similar (Table I). The Palmer method is considered excellent, and these results tend to substantiate Newcomer's contention concerning the accuracy of his method. In addition Robscheit compared the Palmer method with the Van Slyke oxygen capacity method and found that the hemoglobin determinations were almost identical (Table II). The fact that the Palmer, Van Slyke, and Newcomer disk readings were so similar tends to prove that the Newcomer disk method is dependable. In

TABLE II  
*Hemoglobin*

PALMER'S METHOD	AUTHOR'S MODIFICATION
<i>per cent</i>	<i>per cent</i>
75	74
70	70
77	76
77	77
74	73
73	73
74	74
75	74
76	75
73	73
64	64
63	63
76	76
45	45
60	60
84	85
69	69

TABLE III

VAN SLYKE'S OXYGEN CAPACITY METHOD	PALMER'S METHOD	AUTHOR'S MODIFICATION
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
113	113	114
117	118	117
99	98	99
124	124	124
109	108	109

general, with a disk of satisfactory thickness, the Newcomer method is very accurate and is of immense practical value, eliminating the preparation of standard solutions.

*Cohen and Smith Method.*—In determining the hemoglobin by this method, originally 0.20 c.c. of the blood to be examined was diluted with 6 c.c. of 0.1 normal hydrochloric acid. It was found early that the plunger of the colorimeter was often out of the acid hematin solution when the readings were low and only 6 c.c. of 0.1 normal hydrochloric acid was used. For this reason 0.40 c.c. of blood and 12 c.c. of 0.1 normal hydrochloric acid were always used thereafter, with no further difficulties.

Meyer and Butterfield, Newcomer and others claim that a maximal color depth is slow to appear in the acid hematin methods, and that the readings

vary from 2 to 20 per cent if the estimations are made too soon. Newcomer says that the readings can be made safely at the end of forty minutes. Therefore, in the methods involving acid hematin solutions, all readings were made in from six to twelve hours. Berman maintains that this difficulty can be overcome by boiling the acid hematin solution for one minute, allowing it to cool for one minute, and then making the estimation. Considerable criticism of acid hematin methods has arisen on account of the unstable properties of acid hematin standards. Using defibrinated blood and following the technic of Robscheit, or of Cohen and Smith, standards are obtained which remain uniform from four to seven months. If the 0.5 per cent standard is made up every two to three weeks and kept on ice, the readings should be very accurate. In this investigation fresh 0.5 per cent standard solutions were made up weekly, and the 20 per cent stock standard solutions every three months. The Duboseq colorimeter was used exclusively. Robscheit also compared her acid hematin method with the Palmer method and obtained similar readings (Table III).

*Tallqvist Hemoglobinometer.*—This instrument was used more out of curiosity than to determine the real value of the method. The directions were carefully followed with curious results. While the instrument is not accurate, the readings are at least approximate. According to Barker, "If it does nothing more than lead the practitioner to realize the necessity of seeing the color of drawn blood as it appears on filter paper, or on a towel, and to recognize the fallacy of trusting to the appearance of visible mucous membranes, it is in so far, praiseworthy."

#### RESULTS OF THE VARIOUS METHODS OF HEMOGLOBIN DETERMINATION USED

In the first study, represented by the curve in Fig. 2, 199 patients were examined. The hemoglobin values obtained on the Dare instrument were checked against those obtained by the acid hematin method as described by Cohen and Smith. As a result of these findings, further investigations were made, and ninety-one additional patients examined. In this work the Cohen and Smith method is considered the standard, and in the estimation of the accuracy of other hemoglobinometers used, the hemoglobin percentages obtained by these instruments are compared with those obtained by the Cohen and Smith method. The individual readings representing the percentage of hemoglobin calculated in each method are brought out in Figs. 2, 3, 4, and 5.

#### CONCLUSIONS

1. The standard Dare hemoglobinometer is a practical instrument for hemoglobin determinations from 20 to 60 or 65 per cent.
2. Above 70 per cent the hemoglobin estimations on the standard Dare instrument are very misleading.
3. The Newcomer disk method is a unique and practical method of estimating the percentage of hemoglobin in the blood. The percentages obtained are reliable.
4. The Cohen and Smith acid hematin method is accurate and fairly

practical and should be used in the estimation of the percentage of hemoglobin present in all suspected cases of anemia.

5. The special Dare instrument is a decided improvement over the standard Dare instrument.

6. The Tallqvist hemoglobinometer is probably quite as accurate as the standard Dare instrument.

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## LABORATORY METHODS

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### THE DETERMINATION OF URIC ACID IN BLOOD BY THE METHOD OF FOLIN AND WU\*

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BY ROGER S. HUBBARD, PH.D., AND LUCY L. FINNER, B.S. (CHEM.), NEW YORK

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A NUMBER of investigators have recently discussed the determination of uric acid by the method described by Folin and Wu in 1919. Several of them (Jackson and Palmer, 1922; Morris and Macleod, 1922 a; Benedict, 1922; Folin, 1922) have suggested modifications in technic by which it is possible to obtain more color from a given amount of uric acid than could be obtained by the method as originally described. Pucher (1922 a) has stated that part of the uric acid present in blood is carried down with the precipitated protein, and (Pucher, 1922 b) has suggested a method for obviating this difficulty, as has also Folin (1922). Morris and Macleod (1922 a) have pointed out that reduced silver may be introduced with the reagents used for separating uric acid from interfering substances, and have suggested that it be separated as the zinc salt (discussed by Morris in 1916) instead of as the silver salt as recommended by Folin and Wu. Morris and Macleod (1922 b) have also described a reaction noted when potassium oxalate was added to blood; in a certain number of specimens they found higher values after this salt was added. They have attributed this increase to the formation of uric acid from some second compound present in the blood, which, from its chemical behavior, they have designated as a second form of uric acid. Benedict (1922) has discussed the theoretical and practical difficulties involved in carrying out the separation of uric acid from interfering compounds recommended by Folin and Wu, has described a method for the determination of uric acid without such a preliminary separation, and has given a table in which values obtained by this new method and by the method of Folin and Wu are compared with each other and with nonprotein nitrogen determinations done upon the samples of blood. Folin (1922) has also discussed this separation of uric acid from interfering compounds, and described a modification of the technic used for precipitating it.

In discussing the table of comparative results referred to above, Benedict points out that the method for determining uric acid directly in protein-free filtrates tends to give higher values than does the method of Folin and Wu upon blood with normal nonprotein nitrogen content, as would be expected

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\*From the laboratories of the Clifton Springs Sanitarium, Clifton Springs, New York.  
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if the method of separation employed in the earlier method failed to recover all the uric acid present. On the other hand he found that bloods containing increased amounts of nonprotein nitrogen sometimes gave higher results by the method of Folin and Wu than by the new method. He believes that there is some compound, not uric acid, in such bloods which carries through the method of purification and reacts with the reagent used in the method. Many results have been obtained by the method of Folin and Wu in various clinical pathologic laboratories, and it has seemed worth while to examine a series of such results to determine, if possible, to what extent these difficulties have affected the clinical interpretation.

In the series presented here variations in the degree of nephritis as determined by various tests have been used as a standard for estimating the significance of the uric acid determinations. Myers (1921) has recently summarized the work upon the retention of uric acid in this condition, and has concluded that increases in the amounts of uric acid in the blood regularly accompany increases in the amount of urea present. He has shown too that increased amounts of uric acid are found in the blood of nephritic patients when the condition has not advanced far enough to produce measurable increases in the amount of urea present. These findings have recently been confirmed by Czoniczer (1922).

The results discussed here include all of the determinations of blood uric acid on blood drawn before breakfast made in this laboratory during a period of two years and a half following the introduction of the method of Folin and Wu, except determinations carried out on three cases of gout (in which the expected high values were found) and those done on twenty-six cases in which no determinations of urea were made, and on which, therefore, there is not sufficient data available for determining approximately the degree of nephritis present. Total nonprotein nitrogen determinations were done upon the larger part of these twenty-six cases, and the results appeared to agree well with those discussed. No cases of leucemia were studied during this time.

Before proceeding with a discussion of the results certain facts concerning the cases studied and the difficulties recognized in the use of the Folin and Wu method should be mentioned. Most of the specimens were from patients who were considered as possible cases of nephritis, or who were suffering from heart disease or hypertension. In many instances they had been living upon diets low in protein for some time before they were admitted to the hospital, and a comparatively low concentration of urea in the blood was the rule. It has been suggested (Hubbard, 1922 a) that 18 or 20 mg. of urea nitrogen per 100 c.c. of blood should be regarded as the upper limit of normal in the fasting blood in such a series. Of the objections to the method discussed above, the difficulty in reading the color given by such amounts of uric acid as are found in normal blood and irregularities produced by the presence of varying amounts of potassium oxalate were the only ones recognized as markedly influencing the results, and of these the effect of potassium oxalate alone appeared to influence the clinical in-

terpretation of the values obtained. Precautions similar to those suggested by Folin and Wu were used in collecting the blood to insure the presence of a minimum amount of the salt, but it was not always present in the same concentration in all specimens. It was noticed that samples which form their behavior with the protein precipitants seemed to contain an excess of the anticoagulant appeared to contain relatively large amounts of uric acid. In determinations on other samples from the same cases smaller amounts were found when there was not the same reason to suspect the presence of an excess of oxalate. The addition of this salt before the removal of the proteins caused an apparent increase in the amount of uric acid present in some specimens. On the addition of silver lactate to such specimens a larger bulk of precipitate than usual was noticed. These results were in accord with the experimental work of Morris and Macleod, but a different interpretation was given to them than that which those authors have given. It was thought that all of the uric acid was not recovered in some cases when only minimum amounts of potassium oxalate were present, and accordingly the probable borderline between normal and increased amounts of uric acids was placed at the low figure of 2.5 mg. per 100 c.c. (Hubbard, 1923) and a conservative interpretation was placed upon results somewhat in excess of this figure.

TABLE I

UREA NITROGEN		URIC ACID										
RANGE	NO.	RANGE—MG. PER 100 C.C.										
MG. PER 100 C.C.		To 1	1-1.5	1.5-2	2-2.5	2.5-3	3-3.5	3.5-4	4-5	5-6	6-8	8-10
		NO.	NO.	NO.	NO.	NO.	NO.	NO.	NO.	NO.	NO.	NO.
to 12	80	3	10	19	12	6	12	7	6	2	3	0
12-14	65	1	3	9	15	12	9	6	6	2	1	1
14-16	25	0	0	7	4	6	3	1	2	1	1	0
16-18	25	0	3	3	7	2	2	4	2	1	1	0
18-20	7	0	1	3	0	0	0	0	2	1	0	0
20-22	7	0	0	0	3	2	1	1	0	0	0	0
22-25	3	0	0	0	0	1	0	0	1	1	0	0
25-30	5	0	0	0	1	1	1	1	0	1	0	0
30-40	4	0	0	0	0	0	0	1	1	1	1	0
40-50	3	0	0	0	0	0	0	0	1	2	0	0
50-70	8	0	0	0	0	0	0	1	1	2	4	0
70-100	4	0	0	0	0	0	0	0	0	1	3	1
100+	3	0	0	0	0	0	0	0	0	1	1	1

In Table I the different levels of blood urea nitrogen (determined approximately in the way described by Van Slyke and Cullen in 1914) have been grouped together, and the number of cases in each group which showed different amounts of uric acid in the blood given. Inspection of the table shows that in general the percentage of cases which gave high values for the uric acid content of the blood increased as the values of the urea nitrogen increased, but that there were many exceptions to such a statement. Four samples of blood which contained more than 20 mg. of urea nitrogen per 100 c.c. of blood contained 2.5 mg. or less of uric acid, and four more contained between 2.5 and 3 mg. These results cannot be easily explained except on the basis of the failure of these cases, which probably were suffer-

ing from nephritis (the excretion of phenolsulphonophthalein was decreased in four of the eight cases, and was normal in two) to show a retention of uric acid, or of failure to recover all of the uric acid present by the technic used. More cases seemed to show larger amounts of uric acid than the urea content of the blood would indicate, but these cases include mild cases of nephritis in which the urea content is not increased. To determine the percentage of these cases which can properly be classified as cases of early nephritis the figures found by the phenolsulphonophthalein test (Rowntree and Geraghty, 1919) are given in Table II. Frontz and Geraghty (1922)

TABLE II

URIC ACID		PHENOLSULPHONOPHTHALEIN TEST				
RANGE	NO.	NO.	RANGE—PER CENT			
MG. PER 100 C.C.			70+	50-70	30-50	To 30
			NO.	NO.	NO.	NO.
2.5-3	26	24	2	10	10	2
3-3.5	26	16	2	7	6	1
3.5-4	18	10	0	4	5	1
4-5	18	15	1	6	7	1
5-6	7	7	2	1	4	0
6+	6	4	0	0	3	1

Urea nitrogen concentration less than 20 mg. per 100 c.c. of blood.

and Hubbard (1922 a) have shown that a decreased excretion of this dye can be detected in cases of nephritis before an increased retention of urea can be demonstrated. Table II shows that seventy-five per cent of the cases with high uric acid, but low urea concentrations in the blood were tested by the phenolsulphonophthalein test, and more than fifty per cent showed a diminished excretion of the dye, but that some cases with values as high as 5 mg. per 100 c.c. excreted normal amounts. It has been shown (Hubbard, 1922 b) that some cases which show symptoms of nephritis excrete normal amounts of the dye, but show evidences of kidney impairment when tested by the specific gravity fixation test of Mosenthal (1915). Such tests were done upon some of the patients with increased amounts of uric acid in the blood, but normal rates of dye elimination and blood urea concentrations. The results are given in Table III.

TABLE III

URIC ACID		SPECIFIC GRAVITY FIXATION TEST			
RANGE	NO.	NO.	PER CENT AT NIGHT	SP. GR. DIFFERENCE	NEGATIVE
MG. PER 100 C.C.			45 AND MORE	0.010 AND LESS	NO.
			NO.	NO.	
2.5-3	12	7	1	3	3
3-3.5	9	7	4	4	2
3.5-4	4	4	1	3	1
4-5	7	4	3	3	0
5-6	3	2	1	0	1
6+	0	0	0	0	0

Urea nitrogen concentration less than 20 mg. per 100 c.c. of blood.

Phenolsulphonophthalein excretion greater than 50 per cent in two hours.

Inspection of the Table shows that seventy per cent of the cases tested excreted forty-five per cent or more of the urine at night, or showed a dif-

ference between the specific gravities of different specimens of 0.010 or less. Both of these findings are commonly found only in nephritic patients, and therefore it can be said that in only a relatively small percentage of cases which contained increased amounts of uric acid in the blood further evidence of nephritis could not be found by other quantitative or semiquantitative tests, but there were many cases upon whom complete studies were not made.

A study of the uric acid values obtained in this series has shown that increases in the urea concentration of the blood were usually accompanied by increases in the uric acid concentration. In the larger part of the cases which showed increased retention of uric acid but normal concentrations of urea some degree of renal impairment could be demonstrated by other tests, but there were some cases in which such impairment could not be demonstrated; there was no reason for believing that these patients were suffering either from gout or from leucemia. A small number of cases failed to show any retention of uric acid when urea retention was found. The figure regarded as the upper limit of normal was placed at the low value of 2.5 mg. of uric acid per 100 c.c. of blood, and the study has shown that when values between 2.5 and 3.0 mg. were found, slight evidences of nephritis may or may not be given by other tests. In general it may be said that the presence of some degree of nephritis could be demonstrated in about half the cases in which these borderline figures were found.

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# A STUDY OF THE RELATION BETWEEN THE PRESENCE OF INDICAN AND OF UROROSEINOGEN IN THE URINE IN CERTAIN DISEASES\*

BY JACOB ROSENBLOOM, M.D., PH.D., PITTSBURGH, PA.

## I. INTRODUCTION

UROROSEINOGEN<sup>1</sup> was shown by Herter to be indol-acetic acid. Indican is the potassium salt of indoxyl-sulphuric acid. One would think that in conditions of an increased excretion of indican that indol-acetic acid would also be excreted in larger amounts than normal. This study was undertaken to discover if this were so.

## II. METHODS

The indican was tested for by the method described by Rosenbloom,<sup>2</sup> using 10 c.c. of urine from a 24-hour specimen, 10 c.c. Obermyers reagent and 5 c.c. of chloroform, the extent of the bluing of the chloroform determining the gradation of the indican present.

The uroroseinogen was tested for by mixing 10 c.c. of urine with 10 c.c. of C.P. hydrochloric acid, adding five drops of a freshly prepared one per cent solution of sodium nitrite and noting the deepness of the rose-red color that appears when uroroseinogen is present.

Following are the cases studied:

TABLE I

CASE NO.	DIAGNOSIS	INDICAN	UROROSEINOGEN
1.	Diabetes	++++	++
2.	Acute glom. nephritis	++	+++
3.	Gastric ulcer	+	++
4.	Cancer of breast	++	Faint
5.	Typhoid	+++	++
6.	Diabetes	0	+
7.	Pneumonia	++++	++++
8.	Pneumonia	0	+
9.	Chr. cholecystitis	0	+
10.	Bichloride poisoning	+	Faint
11.	Pneumonia	++	++++
12.	Typhoid	0	++
13.	Indicanuria	++++	Faint
14.	Chr. nephritis	+++	Faint
15.	Chr. endocarditis	++	++
16.	Arteriosclerosis	0	+++

## III. CONCLUSIONS

From a study of this table it may be noted that there is no constant relation between the excretion of indican and uroroseinogen. At times with an increased indican there is present an increased uroroseinogen, but however with a persistent indicanuria of marked degree there is often only a faint trace of uroroseinogen present. However occasionally they do parallel each other.

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As they both have the same significance as an indicator of intestinal putrefaction, I would draw attention to the necessity of testing for both substances in the urine when using these tests as an indication of intestinal putrefaction.

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<sup>1</sup>Herter: Jour. Biol. Chem., 1908, iv, 107, 239, 253.

<sup>2</sup>Rosenbloom: New York Med. Jour., 1913, xeviii, 814.

## AN EMERGENCY MICROTOME\*

BY ARTHUR H. GLAISTER, TECHNICIAN, FLORENCE, ALABAMA.

IN the absence of a microtome, passable sections can be made in the following manner:

Place the microscope beside a steady shelf of suitable height, remove the eye piece and fit the draw tube with a cork having a coin cemented on top with sealing wax.

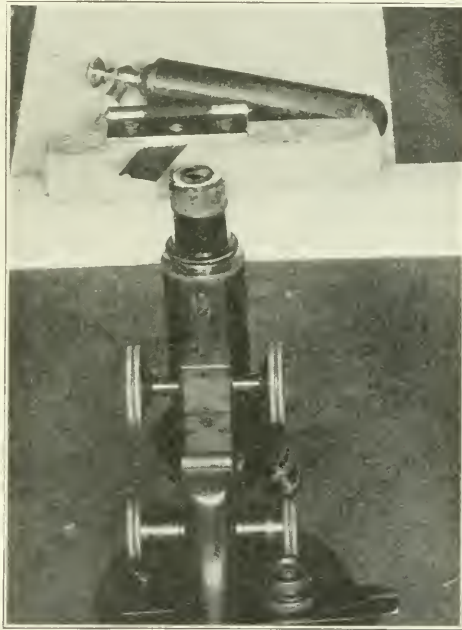


Fig. 1.

For the knife use a safety-razor blade clamped in a folded hinge which is secured to a block of fairly hard wood with wood screws.

Freeze the tissue on the coin with a spray of ethyl chloride and cut by sliding the knife block along a piece of glass secured to the edge of the shelf, adjusting the tissue to the knife with the coarse focus and feeding the desired thickness between cuts with the fine focus.

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# GASTRIC ANALYSIS AND THE CONSTANCY OF THE PERCENTILE RELATIONSHIP AMONG THE TITRABLE FACTORS OF THE GASTRIC SECRETION\*

(PRELIMINARY PAPER)

BY B. S. LEVINE, PH.D., WAUKESHA, WIS.

GASTRIC analysis has failed frequently to be of the desired assistance to the diagnostician, and for this reason it has fallen into ill repute with many a physician, especially since the development of the x-ray technic and the spectacular results it affords. A thorough search of the situation, however, reveals that the fault rests not with the inadequacies of the results of the gastric analyses, but with our inability to interpret the results and to properly associate them with the general clinical conditions. Rehfuß and Hawk have stated justly that it is erroneous to conclude that a certain degree of acidity is indicative of a definite pathologic condition just because a large percentage of cases with such a condition possessed a similar degree of acidity. Many instances have come under the writer's observation in which cases with a total acidity of about 65°, a total free acidity of 45°, and a total physiologically active acidity of 51° were designated by the attending physician as "Hypoaclidity" and HCl preparations were prescribed, whereas others have designated as "normal acidity" cases in which the total acidity was between 22° and 12°, the total free acidity was between 13° and 7°, and the total physiologically active acidity was between 20° and 10°.

Lack of reliable information concerning the proper limits of high and low acidity in normal individuals was another source which added to the confusion prevailing in the gastric analyses. This, however, has been adequately established by Rehfuß and Hawk and their collaborators. It is well known now that there are normal individuals with low acidity as well as with high, and the mere determination of the degrees of the various acidities for this reason adds little of value in the attempt to establish the gastric status of individual cases.

With the view to finding some factors of value in such cases, the writer has carried out gastric analyses on a number of individuals, and prolonged series on the same individuals. The meal used in all cases was the so-called Ewald meal, consisting of a piece of butter-free toast and a glassful of water, and which was removed by siphoning or by the use of aspiration, as the case required, one hour after the ingestion of the meal. The titrable factors were

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determined by titrating 10 c.c. of the unfiltered gastric contents with N/10 NaOH against the following indicators: phenolphthalein, alizarine sodium monosulphonate, and dimethylaminoazobenzol. The titration was performed immediately after the removal of the gastric secretion from the stomach. Like the above-cited investigators, the writer has found that high degrees of acidity are met with in cases whose history and microscopic examination of the gastric content are negative. Low degrees of acidity, at first glance of a seemingly pathologic character, were also obtained from individuals with no pathologic complaints. Individual cases were then followed up from day to day in an attempt to determine the constancy of volume and of the degrees of the various acidities in the same individuals, assuming that in normal cases these findings would be more or less positive, whereas in the cases involving gastric pathology the regularity would be upset. The assumption proved a mistaken one. Volumes and degrees of acidity varied frequently in the same normal individuals sufficiently to render this point valueless for diagnostic purposes.

The relationship which might exist between the titrable factors was then thoroughly studied. After numerous mathematical calculations, the following simple method of reporting gastric titrable factors was adopted. It revealed interesting and instructive information. Total acidity was reported in absolute degrees of acidity as usual, and was also designated as 100.0 per cent. The other factors were reported in terms of absolute degrees of acidity as determined by titration and in terms of their percentage of the total acidity. Over fifty cases have been studied in this way thus far, but only several examples of actual analyses are given below:

## PATIENT A. K. D.

	9-19-'22	9-22-'22	9-23-'22
Total acidity .....	22°—100.0%	12°—100.0%	35°—100.0%
To. free acidity .....	13 — 59.0	7 — 58.3	21 — 60.0
Combined HCl acidity .....	9 — 41.0	5 — 41.7	14 — 40.0
Free HCl acidity .....	13 — 59.0	7 — 58.3	21 — 60.0
To. physiol. active acidity .....	22 — 100.0	12 — 100.0	35 — 100.0
Organic acidity .....	00 — 000.0	00 — 000.0	00 — 000.0

The noteworthy points in connection with the results of the above analyses are the low degrees of the acidities, the great variations manifest in the absolute figures, and the constancy of the percentile relationships. Microscopic findings in this case were negative.

The constancy of the percentile relationship of the titrable factors in the gastric secretion as manifest after the Ewald meal is not a temporary one, but is apparently typical of the individual, as may be seen from the following analyses of gastric content taken at considerably remote dates:

## PATIENT J. K. C.

	12-31-'21	1-17-'22	9-13-'22
Total acidity .....	52°—100.0%	65°—100.0%	60°—100.0%
Total free acidity .....	32 — 61.5	45 — 69.2	40 — 66.7
Combined HCl acidity .....	20 — 38.5	20 — 30.8	20 — 33.3
Free HCl acidity .....	21 — 40.4	31 — 47.3	28 — 46.7
To. physiol. active acidity .....	41 — 78.9	51 — 78.1	48 — 80.0
Organic acidity .....	11 — 21.1	14 — 21.9	12 — 20.0



There seems to be some difference in the Ewald meal administered on dates distantly apart. In such cases the percentile values of the titrable factors occasionally differ somewhat, but the percentile value of the total physiologically active acidity remains constant. The following analyses demonstrate this point:

## PATIENT C. W.

	11-15-'21	9-20-'22
Total acidity .....	44°—100.0%	54°—100.0%
Total free acidity .....	40 — 90.0	33 — 61.1
Combined HCl acidity .....	4 — 9.1	21 — 38.9
Free HCl acidity .....	30 — 70.0	21 — 38.9
To. Physiol. active acidity .....	34 — 79.1	42 — 77.8
Organic acidity .....	10 — 20.9	12 — 22.2

However this may likewise be due to differences in the protein concentration of the gastric secretion itself, and may also prove of diagnostic value.

As far as the work has progressed now, it has indicated that in certain pathologic gastric conditions the constancy in the percentile relationship of the titrable factors is disturbed, and thereby gives warning to the attending physician. Certain deviations from this constancy are observed also in purely nervous conditions, especially in the so-called "anxiety neuroses," but the deviations are not as pronounced and seem to affect the percentile value of total physiologically active acidity only to a slight extent.

On the basis of the work done by us thus far, we have come to the following tentative conclusions regarding the titrable factors of the gastric analysis:

1. That there exists a constant percentile relationship between the titrable factors of the gastric secretion in the normal stomach.
2. That the Ewald meal test is the most convenient and in practically all cases with which the ordinary practitioner has to contend is a sufficient means for the determination of the gastric status of the patient from the physiologic and so-called functional points of view.
3. That high or low acid values may be typical of the individuals under investigation, and, hence, are to be considered normal in such cases, this point being a corroboration of the conclusion of Rehfuess, Hawk, and their collaborators.
4. That variations in the degrees of the acidities even to a marked extent may occur on consecutive days in the gastric contents of the same individuals with no pathologic gastric involvements.
5. That a series of at least three gastric analyses of the same type shall be performed on successive days, and the constancy of the percentile relationship of the titrable factors should be determined before any conclusion or opinion is formed regarding the case studied.

This preliminary paper is given publicity with the hope that it will stimulate interest and further investigation along the line of *the constancy of the percentile relationship of the titrable factors of the gastric secretion*. The detailed report of the work done by the writer in this line will be published in the future.

## THE ESTIMATION OF ALBUMIN IN URINE\*

BY ARMAND J. QUICK, PH.D., PHILADELPHIA, PA.

THE simplest and best known clinical method for the estimation of albumin in urine is that of Esbach<sup>1</sup> which depends on the precipitation of albumin by a picric acid-citric acid reagent in a tube so calibrated that the height of precipitate after standing 24 hours can be directly read in terms of grams of protein per liter. In spite of all criticisms,<sup>2</sup> condemnations, and proposed modifications, the original method is still widely used, although it is well recognized that the results often are not even roughly quantitative. The greatest source of error lies in the tube itself. Since the method calls for an empirically calibrated tube, the specifications of which were never published, the original tube has been copied and recopied with ever accompanying errors so that now a variety of tubes are found on the market which vary greatly in shape and dimensions. The method, moreover, is empirical and takes little account of such factors as temperature and the specific gravity of the urine.

While numerous modifications have been proposed<sup>2</sup> it seems that none offer any distinct improvement either as to increasing its accuracy or its simplicity. With the aim of securing greater accuracy without destroying the simplicity of the procedure the following investigation was undertaken.

Since none of the Esbach tubes available were found satisfactory a 20 cm. tube rounded on the bottom without tapering, and having an internal diameter of 12 mm. was selected.† It was graduated in 0.5 c.c. up to 10 c.c. and further calibrated for 15 c.c.

In order to calibrate the new tube like the original Esbach in grams of albumin per liter. 10 c.c. of urine containing a known amount of egg albumin was transferred to the tube and treated with 5 c.c. of Esbach reagent (1 part picric acid, 2 parts citric acid, dissolved in 100 parts of water). After thorough mixing, the tube was allowed to stand for 24 hours at 21° C. Table I records the reading of the height of the precipitate in c.c. obtained with varying amounts of albumen.

In a similar way two other protein precipitating agents were studied. The first was Tsuchiya's reagent (1 part phosphotungstic acid and 5 parts conc. hydrochloric acid in 100 parts of 95 per cent alcohol) which was recently highly recommended by Pfeiffer,<sup>3</sup> the second was a 10 per cent solution of trichloroacetic acid which, heretofore, has not been used as a substitute for the Esbach reagent. A comparison of the data obtained by the analysis

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†The tubes were kindly furnished by the Kimble Glass Co.

of urines containing known amounts of egg albumin with the different reagents may be made by referring either to Table I or Chart 1.

TABLE I

CONC. OF ALBUMIN G. PER LITER	READING IN C.C.		TRICHLORACETIC ACID
	ESBACH	TSUCHIYA	
0.5	0.6	0.8	0.5
1.0	1.2	1.6	1.1
1.5	1.7	2.4	1.6
2.0	2.2	3.0	2.1
2.5	2.6	3.4	2.6
3.0	3.0	3.8	3.2
3.5	3.3	4.2	3.7
4.0	3.6	4.5	4.2
4.5	3.9	4.8	4.6
5.0	4.2	5.0	5.0

Specific Gravity of urine 1.013-1.015. Temperature 21-22° C.

The effect of temperature and of the specific gravity of urine was studied next, since these are the most important variable factors in the analysis. In studying the influence of temperature the albumin was precipitated in the usual way, using the three different reagents. One set of tubes was allowed to stand for 24 hours at room temperature (21-22° C.), another at 25° C., and the third at 10-11° C. The results are recorded in Table II.

TABLE II

VARIATIONS OF HEIGHT OF PRECIPITATE WITH TEMPERATURE

REAGENT	G. OF ALBUMIN PER LITER		
	10-11° C.	21-22° C.	26°
Esbach	3.2	2.4	2.0
Tsuchiya	5.	2.9	2.8
Trichloracetic	3.3	2.9	2.8

Urine contained 2.9 parts albumin per liter. Sp. G. 1.020.

To determine the effect of specific gravity on the height of the precipitate, a series of urines of different specific gravities were used, and a known amount of egg albumen was added to each specimen. In order to get the high specific gravity values, urea was added, while the lower values were obtained by dilution. The results are recorded in Table III.

TABLE III

EFFECT OF SPECIFIC GRAVITY ON THE HEIGHT OF THE PRECIPITATE

Reagent	G. OF ALBUMIN PER LITER					
	SPECIFIC GRAVITY					
Reagent	1.003	1.012	1.017	1.022	1.028	1.045
Esbach	3.2	2.5	2.3	2.4	2.0	2.4
Tsuchiya	3.9	3.5	3.0	2.9	2.8	2.9
Trichloracetic acid	2.8	2.9	2.8	2.8	2.8	2.7

Temperature 20° C., conc. of albumin 2.9 g. per liter.

The results from all the experiments clearly point to the superiority of trichloracetic acid as a precipitating agent. In the first place the height

of the precipitate is very nearly directly proportional to the concentration of albumin within the limits of .05-.5 per cent; consequently there is less error in the higher readings than with either of the other two reagents. Fortunately, the Esbach and trichloroacetic acid curves are nearly identical up to 0.3 per cent albumin so that the two can be used interchangeably for urines with low concentration of albumin. The greatest advantage in the use of trichloroacetic acid lies in the fact that the effects of temperature and of the specific gravity of urine are reduced to a minimum. The precipitates obtained both with the Esbach's and Tsuchiya's reagent are effected by temperature, and the Esbach especially gives readings which are too low if the temperature is much above 20° C. Variation of specific gravity decidedly influences the volume of precipitate with Tsuchiya's reagent, the reading taken at a specific gravity 1.010-1.013 being about 30 per cent higher than at 1.020-1.025.

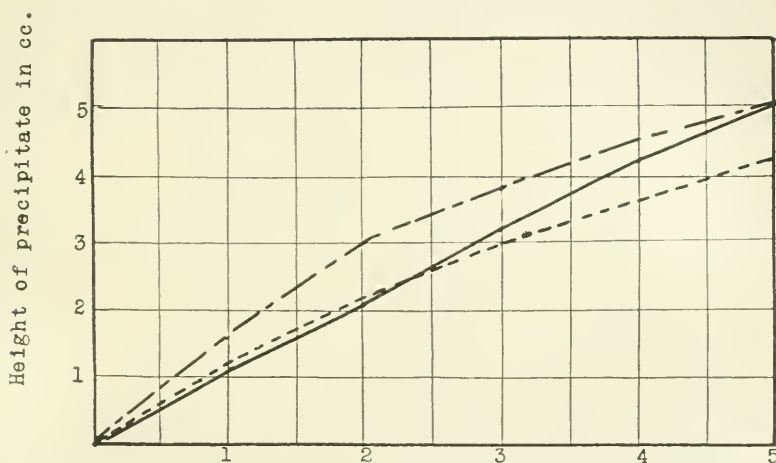


Chart 1.—Grams of albumin per liter. - - - - Esbach - . - . Tsuchiya ——— Trichloroacetic acid.

The method was next applied to pathological urines. The procedure was carried out as follows: 10 c.c. of urine were transferred to the tube, previously described, 5 c.c. of the reagent added, the tube stoppered and inverted 10 times to insure complete mixing. After allowing the tube to stand at room temperature for 24 hours the height of the precipitate was read and converted to grams of albumin per liter by means of Table I. In the case of urines containing more than 5 gm. of albumin per liter, only 5 c.c. of urine were taken and diluted with 5 c.c. of water.

Alkaline urines were acidified with conc. hydrochloric acid until acid to litmus. Both Tsuchiya's reagent and trichloroacetic acid are sufficiently acid to effect complete precipitation in all but exceedingly alkaline urines, but with Esbach's reagent it is necessary to neutralize carefully excess alkalinity. The values obtained were compared with the more accurate coagulation method which was carried out in the following manner: 50 c.c. of urine was slightly acidified with acetic acid, and heated on a water-bath



until the coagulation of protein was complete. The precipitate was removed by centrifuging and after thorough washing was analyzed for nitrogen by the Kjeldahl method. By means of the factor 6.25, the grams of nitrogen found were calculated in terms of grams of protein. By using trichloroacetic acid and following the method as outlined, it is possible to obtain results which are more accurate than those obtained by the use of either Esbach's or Tsuchiya's reagent. It might be remarked in passing that fresh urine should be used. The albumin readily undergoes decomposition if the urine is not well preserved, and even in well preserved specimens of urine, the albumin gradually undergoes changes so that none of the determinations mentioned will give satisfactory results.

TABLE IV  
ANALYSIS OF URINES  
Reading, g. of albumin per liter

ESBACH	TSUCHIYA	TRICHLORACETIC ACID	GRAVIMETRIC	SPECIFIC GRAVITY
2.6	3.2	2.7	2.7	1.016
0.8	0.7	0.6	0.8	1.012
4.5	3.2	3.2	3.2	1.010
1.0	1.0	0.9	0.8	1.012
2.6	2.6	3.0	3.5	1.010
2.6	3.0	3.2	3.2	1.016
1.2	1.5	1.1	1.1	1.010
9.6	10.0	7.0	6.6	1.006
4.3	3.2	2.8	3.1	1.010
1.8	3.5	2.0	1.8	1.013
1.0	1.4	1.0	1.1	1.005
7.6	10.0	6.8	6.7	1.020

#### SUMMARY

1. A comparative study of the modifications of the Esbach method for the determination of protein in urine has been made. Since the available Esbach tubes were unsatisfactory, a new tube was designed and described. Esbach's reagent, Tsuchiya's reagent, and a 10 per cent trichloroacetic acid were studied as precipitating agents. The influence of temperature and of specific gravity of urine were determined.

2. A 10 per cent solution of trichloroacetic acid as a precipitating reagent was shown to yield the most satisfactory results.

The author wishes to express his thanks to Dr. A. D. Waltz for furnishing the pathologic specimens of urine.

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## A CLINICAL TEST FOR LIVER FUNCTION\*

By EMIL BOGEN, CINCINNATI, OHIO

EVIDENCE is rapidly accumulating that the removal of phenoltetrachlorphthalein from the blood stream, as first shown by Abel and Rowntree in 1910, is an index of the functional efficiency of the liver. Much credit is due to Sanford M. Rosenthal for developing a reliable method for the determination of this excretion, and for securing practical standards for the interpretation of the results. Unfortunately, the repeated withdrawals of relatively large amounts of blood, the danger of complete nullification of the readings by the slightest traces of hemolysis, the necessity for preparing fresh standards for each test made, and the consequent difficulty in preparing enough standards for accurate readings, are factors which retard the general adoption of this very ingenious and valuable procedure.

In order to make this test comparable in simplicity and ease of execution with the well-known phenolsulphonephthalein test for kidney function, the

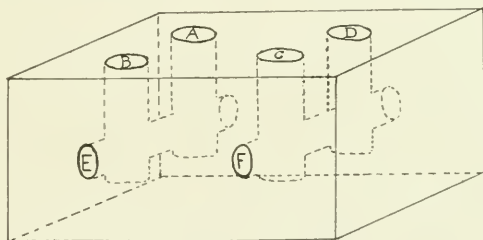


Fig. 1.

method of bicolorimetric comparison with permanent standards of a single sample of blood taken one hour after the injection of the dye was attempted, and the following modified technic was found, in a series of cases at the Cincinnati General Hospital, to give eminently satisfactory results.

The patient is weighed and the dosage determined on the basis of five milligrams of phenoltetrachlorphthalein (or 0.1 c.c. of the sodium phenoltetrachlorphthalein solution put up by Hynson Westcott & Dunning of Baltimore, Md.) for each kilogram of body weight. This dose is drawn up into a 25 c.c. Luer syringe, and sterile physiologic salt solution added to the 25 c.c. mark, and is then injected slowly through a narrow bore needle into a large vein on the right arm, using a tourniquet if needed to bring out the vein, but releasing it before the injection is commenced, and taking great care that the needle is freely in the vein, so that no extravasation may occur.

Exactly one hour later five to ten c.c. of blood is taken from the left arm, with a dry needle into a clean dry test tube, the blood is allowed to clot and the serum to separate out. We have been able to secure clearer sera,

\*From the Department of Medicine, Cincinnati General Hospital.  
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with less danger of hemolysis, by allowing the blood to clot with the test tube in a slanting position, and aspirating the clear serum that separates after several hours by means of a long narrow syringe such as is used in giving tuberculin. The serum obtained is divided into two small test tubes, 1.5 cm. in diameter, and two drops of ten per cent sodium hydroxide are added to one tube to bring out the color of the dye, which remains absolutely colorless without this alkalization in the other tube, thus serving as a control, and the percentage of the dye present is determined by comparing with a set of standard solutions of the dye, through a two-color colorimeter.

A simple bicolorimeter is made as follows: A block of wood 2" by 2" by 2" is taken and four holes are bored perpendicular to one surface just large enough to admit easily the small test tubes used to a depth of three or four centimeters, as shown in Fig. 1, A, B, C, and D.

Two holes are then bored horizontally exactly through these two pair of holes, with slightly smaller diameter, (Fig. 1, E and F) so that, by looking through one of these latter holes one might see through the two tubes standing in the corresponding vertical holes. A piece of ground glass or translucent paper is fastened over the back of the box, to facilitate the readings.

An empty tube is placed in hole A, the alkalized serum in hole B, the control serum without alkali in hole C, and the standard solutions are then successively placed in hole D until one is found which, on looking through hole F, will yield the same color obtained by looking through hole E.

The standard solutions are made by first making a solution containing ten milligrams of dye in 100 c.c. of distilled water, and then making dilutions of 1, 2, 3, 4, 5, 6, 8, 10, 12½, 15, 17½, and 20 per cent of this first standard with distilled water containing a few drops of 10 per cent sodium hydroxide to bring out the color, and placing these diluted standard solutions in twelve small test tubes, 1.5 cm. in diameter, which are then sealed and marked and kept as permanent standards.

The following table is included to indicate the clinical significance of the one hour readings in a series of cases so tested at the Cincinnati General Hospital.

Erythema multiforme	1%
Pleurisy	1½%
Arsenic poisoning	4%
Carcinoma, abdominal	5%
Carcinoma of liver (?)	7%
Banti's Disease (?)	8%
Cirrhosis of liver	8%
Toxemia of pregnancy	10%
Cirrhosis of liver	10%
Catarrhal Jaundice (?)	14%
Cirrhosis of liver	20%
Cirrhosis of liver	22%

Dr. Rosenthal reports 37 cases, with readings at one hour as follows:

Carcinoma of liver	9-18%
Cirrhosis of liver	7-20%
Acute Hepatitis	20-22%
Liver abscess	9-10%
Toxemia of pregnancy	12%
Normals	0- 1½%
Nonhepatic diseases	1- 3%

We may infer from these figures that a reading of less than three per cent definitely testifies to a normally functioning liver, that from 4 to 8 per cent indicates partial hepatic insufficiency, and that a retention of over 8 per cent of the dye an hour after injection represents a pronounced liver impairment.

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## A USEFUL URINARY FINDING IN THE DIAGNOSIS OF HYDRONEPHROSIS AND PYONEPHROSIS\*

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BY JACOB ROSENBLOOM, M.D., PH.D., PITTSBURGH, PA.

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IN three cases of pyonephrosis and in two cases of hydronephrosis, I have found globules of fat in the urine. I think the reason this finding has not been described before is due to the fact that in examining urine microscopically, the sediment is obtained by centrifuging or by allowing the urine to stand until a deposit forms. Fat globules being lighter than urine, come to the surface under these conditions and one must examine the surface layer of the urine to detect the same.

These globules are usually large and strongly refractile and stain black with osmic acid and red with Sudan III. They are also soluble in ether.

It will be recalled that fat in the urine, the so-called "lipuria" is always pathologic, as no free fat is found in normal urine. One must be sure that the fat is not due to contamination from a lubricant used in the catheterization or from the container. There also occurs the so-called alimentary lipuria due to too large an amount of fat in the diet. Webster states that pathologically it has been observed in various cachectic conditions, in crushing injuries, especially of the bones, in eclampsia, in chronic heart disease, fatty tumors, diabetes mellitus, tuberculosis, various affections of the pancreas and liver, nephritis, and after the use of various general protoplasmic poisons. In these cases the blood may also contain an excess of fat. It would be interesting to know if there is present an increased amount of fat in the blood in hydronephrosis and pyonephrosis.

There also exists the parasitic cyturia due to filaria and the nonparasitic cyturia of unknown origin and a lipuria due to inunctions of oil.

In one of the cases mentioned in this paper the fat droplets were only found in the urine obtained on catheterization of the diseased kidney. The catheterized urine from the normal kidney was free of the fat droplets.

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## EDITORIALS

### *The Possibility of Extending Human Life*

UNDER the above heading Dr. Louis I. Dublin, statistician for the Metropolitan Life Insurance Company, presented to the Harvey Society of New York in December, 1922, a most valuable paper. In the first place, Dublin points out that the mortality statistics which are now at our disposal are in no way satisfactory. They are, very properly called, crude death rates, and are likely to lead us into false conclusions. Age has a great influence on death rates and when we compare the mortality of New York City with that of Seattle this must be taken into consideration. If there are more babies and more old folks proportionately in New York City than there are in Seattle, quite naturally the death rate in the latter will be smaller than in the former, since the highest mortality is in the two extremes of life.

The article discusses present time longevity. The first reliable statistics along these lines are those for England and Wales covering the period between 1838 and 1854. At that time the average length of life in these countries was nearly forty-one years. During the following decades there

was a constant gain. The seventh table covering the period from 1910 to 1912 gave an average extension to fifty-three and one-half years. This shows a gain in the interval of seven decades of twelve and one-half years. On the European continent the best historical data are for Sweden. The first table covers the period from 1816 to 1840 and shows an expectation of life of forty-one and one-half years. From 1901 to 1910 the expectation of life in Sweden had increased to fifty-five and three-fourths years, thus showing a gain of fourteen and one-fourth years in an interval of eight decades. In our own country the first reliable table came from Massachusetts in 1855. At that time the expectation of life in Massachusetts was about forty years—the same as in England and Wales. By 1910 the Massachusetts figure had increased to fifty-one years, showing a gain of eleven years in a half century. By 1920 the expectation of life in Massachusetts had reached fifty-five years, or a gain of more than fifteen in sixty-five calendar years. In the Registration Area of the United States there has been an increase of six years between 1901 and 1920.

Turning to the mortality in age groups, Dublin says that the infant mortality in the Registration Area dropped in 1921 to 76.0 per 1,000 births. In New Zealand in 1920 it was below 50.0, and there are a large number of cities in the United States, some of good size, where the infant mortality at the present time is below 40.0 per 1,000 births. Dublin assumes that it is entirely feasible to reduce the infant mortality to 38.2 per 1,000 births; indeed, he thinks that this is not an unreasonable figure and he is inclined to the opinion that the reduction might be still greater if more attention is paid to the prevention of infant mortality during the first month. The detection and treatment of syphilis in the mother, the control of the toxemias of pregnancy, and the provision for skillful obstetrics would do much for the mothers and would greatly reduce the death rate during the first month of life. Dublin is inclined to think that an infantile death rate of 20.0 per 1,000 births is possible, but he has assumed the higher rate of 38.2.

This author assumes a death rate during the second, third and fifth years of life of between 34.0 and 40.0 per cent of the actual mortality rates as shown in the census of 1910, and he assumes the possibility of reaching a mortality rate of 4.2 per 1,000 for the third year of life. In New Zealand nearly fifteen years ago this rate was 5.3. During the third year of life virtually two-thirds of the entire mortality is due to such infections as typhoid fever, diarrhea, measles, scarlet fever, whooping cough and diphtheria, all of which are more or less under human control.

Dublin assumes that during the period between ten and sixty the mortality can be reduced to one-half the figure prevailing in 1910. He says that his assumed values are for the most part only 20.0 per cent below those of New Zealand in recent years. In that country, as in ours, there is still much preventable mortality from tuberculosis in the working period of life. There is also marked mortality from occupational and other accidents, most of which might be avoided. Dublin says that his assumed rates between ten and sixty are very close to those attained by the best life insurance companies

in their current ordinary experience; indeed, at some ages the actual insurance experience is even lower than the values here named. An investigation into the mortality of a group of ordinary policyholders of the Metropolitan Life Insurance Company who have availed themselves of the privilege of examinations over five years ago showed at the end of the period a mortality 28.0 per cent lower than that of the best control group used in comparison.

Since 1911 tuberculosis has declined 50.0 per cent among those insured in the Metropolitan Company and almost as much in the general population of several progressive cities. Not only is this disease rapidly declining in mortality, but the peak of maximum rate by age is constantly being pushed forward into the later years of life. Unfortunately, we are not doing so well with the diseases which depend upon personal hygiene and the general care of the human body. Death rates from the so-called degenerative diseases, like those of the heart and kidney, and other diseases affecting middle life and old age, are not declining. We are making little headway along these lines. These diseases cause heavy losses to the community, because they involve those still in their prime and at the height of their productivity. Heart disease, Bright's disease, and cerebral hemorrhage curtail the expectation of life about four years. Further reduction in the incidence of infectious diseases, such as typhoid fever and diphtheria will bring important results so far as these degenerative diseases are concerned. It is reasonable to expect that an appreciable part of the 50.0 per cent mortality reduction postulated by Dublin in his hypothetical life table will be accomplished through these means. Much good will be done during the productive period of life by improving conditions under which individuals work. Occupational accidents are responsible each year in the United States for at least 15,000 deaths; most of these among men in their prime. In his conclusions Dublin makes no allowance for improvement in the possible saving of life from cancer; neither does he assume that there will be any marked decrease in senile diseases.

From his conclusions, Dublin thinks it within reason to look forward to the prolongation of human life in this country, which is now about fifty-five years, to sixty-five years. The total death rate would be reduced from 13.0 to 8.6 per 1,000. In other words, an extension of the expectation of life by ten years is within the range of accomplishment. The greatest reduction would be in the infant mortality, which would be to one-third that now existing. A reduction during the first year of life, such as provided for in the table, would mean a saving of 126,000 infants. During the first five years of life there would be 171,000 children saved. Between the ages of ten and sixty a reduction, regarded as reasonable, would save 250,000 lives. These figures show strikingly how great are the stakes involved in the future of medicine.

It should be stated that Dublin has figured out these possibilities without calling to his aid any great medical discovery. If an advance could be made in the treatment of heart disease and kidney disease comparable with that recently made in the treatment of syphilis the expectation of life might be considerably advanced above the figures given.

—V. C. V.



### *Ray Therapy for Deafness and Tinnitus*

A PROMINENT roentgenologist has referred to the x-ray treatment of defective hearing as the latest and favorite indoor sport among roentgenologists. The first serious attempt to treat ear conditions by this method was made by Joulin, who in 1908, reported results in ten cases of otosclerosis. There was some improvement in the tinnitus and some apparent improvement in voice perception. No improvement could be demonstrated by objective tests. The next observer, Ortloff, in 1913, published his conclusions on the treatment of ten cases of tinnitus and deafness, in whom he had observed slight improvement, invariably followed by relapse. Siebenman, after a study covering a period of several years, concluded that the results are only in a small degree encouraging. In this country, Stokes in 1920 reported startling achievements with this method of treatment. Since then great numbers of individuals with tinnitus and deafness from whatever source have received varying amounts of x-ray treatment.

Tinnitus may accompany a number of pathological processes, but it usually indicates some local organic change such as labyrinthine disease, suppurative otitis media, tuberculosis, disease of the auditory nerve, or otosclerosis. Although there are many theories the cause of otosclerosis is not known. In the later stages there is always considerable associated bony deposit around the articulation of the stapes. Once it has occurred, this proliferative change can scarcely be alleviated by x-ray treatment. If the original cause of otosclerosis be amenable to ray therapy, we might anticipate some success in delaying or in stopping the progress of the disease. Little more however could be accomplished.

Raynal suggests that the x-ray treatment of otosclerosis be supplemented by repeated catheterization of the Eustachian tube, to ultimately produce improved mobility of the ossicles.

Tinnitus, on the other hand, is often due to chronic catarrhal otitis media, which in turn results from obstruction or other disease in the nasopharynx. It is conceivable that removal of the latter with resultant greater patency of tubes and improved drainage of the middle ear may give relief from head noises. The greater the chronicity with its resulting changes, the less will be the probability of improvement.

Kinney has found that in cases associated with disease in the nasopharynx, forty per cent obtained permanent decrease in the tinnitus and marked improvement in the hearing, both subjectively and to objective tests. Although improvement was sometimes noted in otosclerosis and in chronic otitis media, the results were on the whole less satisfactory.

Jarvis classifies the cases of impaired hearing treated with the roentgen ray as follows. The first class includes children and young adults whose impairment is often explained by the presence of a mass of adenoid tissue in the nasopharynx. The second includes individuals usually between the years of twenty-five and fifty who are subject to frequent head colds, complain of a more or less constant catarrhal discharge from the throat, and



at frequent intervals of a stuffiness in the ears with accompanying deafness. They frequently complain of tinnitus. In the third class are those, usually advanced in years, whose chief symptom is deafness.

In the first class obstruction to respiration is the prominent symptom. In the second throat symptoms exist, while in the third the complaint is only of impaired hearing. Jarvis obtained best results in class two. In them he often finds large middle and inferior turbinates and lymphoid hypertrophy running up either side of the pharynx behind the posterior tonsillar pillars. Relief from tinnitus in the second group was quite constant. This was not uniformly the case in group three.

McCoy, following Stokes' method of treatment, obtained marked improvement in audition in nine out of thirty-five cases of chronic catarrhal otitis media, in none out of two cases of chronic purulent otitis media, in one of two cases of residual purulent otitis media and in two out of six cases of otosclerosis. In some additional cases there was slight improvement, more marked among those suffering from chronic catarrhal otitis media.

He observed a greater patency of the Eustachian tubes in those cases previously affected with a tendency to stenosis thereof. Two individuals believed that they had experienced a decided improvement in the sense of smell. Tinnitus, which was present in nearly all, was stopped in six and alleviated in ten.

Several workers have experienced no satisfactory results, particularly as far as objective tests for improvement were concerned. Hickey, who has treated a number of individuals, found no objective benefit, although many seemed to think that they were some better. Law reports similar results and remarks on the difficulty of making the voice tone of equal intensity twice in succession, especially when one is anxious that the patient should hear better. Also there is a certain psychological element in the patient's attitude. Fowler again found no objective improvement although the patients were usually of the opinion that they could hear better.

Raynal reports considerable relief from attacks, without complete cure, in a case of Meniere's disease.

The work of Kinney and of Jarvis, who in their classifications, have brought out the importance of nasopharyngeal conditions in those cases successfully treated, will aid in obtaining results, by enabling the physician to select such cases as may *a priori* be expected to respond satisfactorily.

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—W. T. V.

### *A Brief Sketch of the Early History of Industrial Hygiene*

ANCIENT Greek and Roman medical men were not ignorant of the influence of occupation upon the causation of disease. According to Pliny, it was the custom even long before his time, for those working in metals, especially in lead, to protect themselves from inhaling the dust by wearing respirators. In 1557 Agricola published a book, entitled, "*De re metallica*" in which he pointed out the fact that workers in dusty occupations are prone to asthma and tuberculosis. In 1700 Ramazzini, of Padua, wrote most learnedly and extensively concerning occupational diseases. In his book, "*De morbis artificum diatriba*," he dealt with dusty occupations and showed that those passing their lives in such occupations furnish an unusually large percentage of cases of tuberculosis. Plumbism, with its most marked signs and symptoms, has been recognized and its cause correctly determined from the earliest historical times.

In the reign of Elizabeth (early seventeenth century) Parliament enacted a law permitting persons who desired to do so to establish houses in which apprenticed children might be instructed in spinning and weaving. This was actually the beginning of the factory system in England. Orphans and other destitute children were apprenticed to the owners of these primitive factories. Children of all ages were employed in these institutions and in some at least, the working hours covered fourteen of each day. It seems that the framers of this early law were actuated, in part at least, by good motives. They desired to give to orphans and other destitute children an opportunity to earn a living. Long before this time spinning and weaving had occupied a large proportion of the time of the industrial poor, but this work was done in small, crowded cottages and often in cellars.

The poor-law act of 1601, whereby apprenticed children could be instructed in spinning and weaving, gradually grew into the great cotton mills of England. In the early days these mills were located in the country where water power could be obtained. The discovery of the steam engine and its use in factories, which occurred about 1785, led to the rapid growth of such manufacturing centers as Manchester and greatly complicated provision for sanitation in the factories. The imports of raw cotton into England stood in 1775 at 5,000,000 pounds. By 1831 this had grown, according to Hope, to 273,000,000 pounds.

From the beginning of the schools for instruction in spinning and weaving, apprenticed children were frequently exploited in a shameful way. They were fed niggardly, housed when not at work under insanitary surroundings, and during working hours which, as we have seen, often extended to fourteen or fifteen a day, they were densely crowded, subjected to infection, and exposed to an environment in which they could not retain health nor resist disease. It was not, however, until 1784, nearly 200 years after the system had been inaugurated that serious complaint was made. This was first voiced by Percival, who reported infectious outbreaks among children employed in certain factories in Lancashire. For eleven years

Percival continued his criticism of the insanitary conditions under which apprenticed children lived and worked in the cotton mills. In 1795 Percival and associates constituted themselves into a local board of health and demanded that there should be legislative protection for the children employed in factories. In 1802 Peel secured the passage of a bill under the title of the "Health and Morals of Apprentices Act." This Act forbade the employment of children for more than twelve hours a day, ordered that provision for elementary education should be made, that the children should be supplied with sufficient clothing, and that the factories should be properly ventilated and whitewashed at least twice a year. The Act, however, was not a success, for two reasons. In the first place, it created a board of inspectors or visitors, which board consisted of two, one of whom should be a justice of the peace and the other a clergyman. These boards did nothing, largely because their members knew nothing concerning the sanitary conditions of factories or the effects of insanitary conditions upon health. In the second place, the Act was confined to the protection of apprenticed children. It was very easy for the owners of these factories to get rid of their apprentices and introduce in their places "free children." They found parents willing that their children should live under any conditions, provided their work contributed to the support of the family. It was not until 1815 that Peel secured the passage of an act limiting the working day to ten hours, applicable to all children, apprenticed and free, employed in cotton mills. In 1832 Thackrah, a Leeds surgeon, published his book, "The Diseases of Tradesmen," which discussed the relation between occupation and disease. In 1833 an act was passed providing for industrial inspection by qualified men, employed by and under the direction of the general government board of health.

It must not be inferred from what we are saying that all mill owners were disregarding of the welfare of their employes. There were some who were ready, even at a financial sacrifice, to improve the sanitary conditions of their factories, restrict the hours of child labor, and provide for elementary education. In 1842 Parliament passed an act excluding women and children of all ages from working in the mines, and two years later, provision for inspectors was extended to all textile factories and a medical examination of all under sixteen years of age was required before employment. The same act prohibited the employment of children under eight years of age and restricted female labor in cotton mills. The introduction of steam-driven machinery had greatly increased the danger of accident and provision for wire guards was made. In 1860 a bill was passed placing bleaching and dyeing works under the factories act. This bill, however, was defective and did not become beneficially operative until ten years later. Special provision was made against the dangers in glazing pottery and in the manufacture of matches. Strange to say, no effective legislation for protecting the workman engaged in the manufacture of white lead was enacted until 1883, and it was not until 1891 that special provision was



made applicable to every industry which, in the opinion of the Secretary of State, might injuriously affect the health of workers.

After the factories had received fairly satisfactory attention so far as the health and welfare of workers were concerned, there grew up, first in the east end of London, what has been known as the "sweat shop," the evils of which have extended to every manufacturing city in the world. Most of the industrial acts had defined a factory as any place in which fifty or more workers are employed. In the sweat shop the provisions of these laws were avoided by the performance of labor in the home. The Workman's Compensation Act, which became a law in England in 1897 and in this country by the several states later, has been of substantial benefit to the health of the working man; indeed, after trial it seems to be approved by the majority of employers.

In this country we have closely followed England in the development of industrial hygiene. In former times the manufacturer supposed the more hours a day his employes worked the more he was getting for his daily wage. The part played by fatigue was unknown and not even suspected by the manufacturer of fifty years ago. We may quite safely predict that the required working hours in our manufacturing establishments will be further decreased and that the conditions of life under which work is done will be improved.

—V. C. V.

## BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

### *Sollmann's Pharmacology\**

THE second edition of this book continues the high standard set by the first. It is arranged with two sets of type, large for general discussions and small for more detailed information. In this way each drug may be studied either minutely or with regard only to the prominent characteristics. Side headings are found at the beginning of nearly every paragraph. As a consequence of these two methods of treatment the book is most valuable for reference work. At the same time, the author has been forced thereby to discard attempts to make the discussions rhetorically sequential. There is usually a slight break in the line of thought at each side heading. From the nature of the work this could scarcely be avoided.

The work is splendidly done throughout. Among the chapters dealing with newer subjects are particularly those on colloidal solutions, electrolysis, osmosis, synthetic drugs, anaphylaxis and the various war gases. The author's

\*A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology. By Torald Sollmann, M.D., Professor of Pharmacology and Materia Medica in the School of Medicine of Western Reserve University, Cleveland. Second edition, entirely reset. Cloth. Price \$5.50. Pp. 1006. Philadelphia and London. W. B. Saunders Company, 1922.



discussion of the pharmacologic action and therapeutic use of alcohol is of particular interest at this time.

A comprehensive bibliography is arranged at the end, and thereby does not interfere with the reading.

### *Microscopic Examination of Foods and Drugs†*

SOLLMANN, in his manual of Pharmacology writes, "The appearance, size and arrangement of the cells and their inclosures (starch grains, etc.) is often the most important aid in identifying a drug, particularly when in the form of powder. The pharmacopeias therefore include microscopic descriptions, whenever these are important."

Greenish's book deals with this method of examination.

Written primarily for those interested in pharmacy and drug and food adulterants, it deals entirely with the microscopic examination and identification of starches, hairs, fibers, spores, glands, woods, stems, leaves, flowers, bark, seeds, fruits, rhizomes and roots. There is a chapter on the adulterants of powdered foods and drugs and a general scheme for the microscopic examination of powders. At the end of each heading the author describes a method for the identification of an unknown powder. The survey of the subject is most comprehensive.

### *Modern Microscopy\**

THE first ninety-one pages of this book deal with the microscope, its construction and use. The physics of light, etc., is discussed in great detail. Various types of microscopes are described. The second portion deals with the use of the microscope in medicine, public health, in the study of tropical diseases, in histology, geology, engineering, agriculture, natural history, etc. But a few pages are devoted to each. The last chapter describes methods of mounting common objects for examination.

There can be no doubt but that when the book was first written in 1893, and the microscope was still much of an innovation, such a book would have been extremely valuable. However, as time goes on, the field of usefulness for a scientific book dealing with the present day microscope per se is gradually dwindling.

†The Microscopical Examination of Foods and Drugs. By Henry G. Greenish, F.I.C., the University of London; Director of the Pharmacy Research Laboratory; Docteur (H.C.) De L'Universite De Paris; Hamburg Gold Medallist, 1917; Joint Editor of the British Pharmacopeia, 1914; Late Member of the Board of Examiners of the Pharmaceutical Society of Great Britain; Late External Examiner in Materia Medica and Pharmacy to the University of Birmingham. A Practical Introduction to the Methods Adopted in the Microscopical Examination of Foods and Drugs, in the Entire, Crushed and Powdered States. Third edition. Cloth. Price \$4.50. Pp. 386 with 209 illustrations. Philadelphia: P. Blakiston's Son & Co., 1012 Walnut Street. 1923.

\*Modern Microscopy. A Handbook for Beginners and Students. By M. I. Cross and Martin J. Cole. Lecturer in History at Cooke's School of Anatomy. Fifth edition. Revised and rearranged by Herbert F. Angus. Cloth. Price \$3.50. Chicago Medical Book Co., Chicago, Ill. Pp. 315 with numerous illustrations and diagrams. 1922.

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## ORIGINAL ARTICLES

### ON THE NORMAL URINE SUGAR CURVE IN NORMAL INDIVIDUALS, BORDERLINE DIABETICS AND SEVERE DIABETICS UNDER INSULIN TREATMENT\*

BY IRVINE H. PAGE

INCIPIENT diabetes may be controlled by such simple therapeutics as diet, therefore its early recognition is of paramount importance.

The following investigation was undertaken with a view to determine a method of recognizing this condition that would involve few technical difficulties, one that was delicate enough to detect small fluctuations in the ability of the individual to prevent sugar wastage, and one that would indicate the state of sugar metabolism for continuous and prolonged periods of time—not momentary states as pictured by blood sugar methods.

The Benedict-Osterberg normal urine sugar method<sup>1</sup> offered such a means, being delicate enough to detect the normal fluctuations in urine sugar and yet flexible enough to determine pathological sugar excretion. Believing that in incipient and borderline cases of diabetes a pathological sugar wastage might occur only at certain intervals—possibly of extraordinarily short duration—we have adopted the plan of analyzing specimens taken at hourly intervals from 7:00 A.M. to 7:00 P.M. and the combined 12 hour specimen of the night. We believe our results justify what might seem to be an excessive number of determinations. The question of diet, nervous influences and other factors will be considered in due order. The investigation covers the 24 hour sugar curves of (1) the normal; (2) borderline diabetics; (3) diabetics on insulin treatment; (4) one case of renal diabetes; (5) one case of borderline diabetes on insulin treatment, and (6) hyperthyroid cases.

\*From the Eli Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, Indiana.

## METHODS

The patient is given 13 one hundred c.c. bottles containing a few c.c. of toluene, a 250 c.c. graduate and a list of the foods to be eaten on the test day. Instructions are given to empty the bladder at 7:00 A.M., to collect and measure the urine hourly to 7:00 P.M., saving a sample for analysis. The combined 12 hour night specimen is also saved. All specimens must be kept on ice. The patients must be cautioned that the time of voiding, amount, etc., must be conscientiously recorded. These samples are then taken immediately to the laboratory for analysis. No difficulty will be encountered in getting the patients to void hourly. The test should be started not later than 8:00 A.M. The holding of urine in the bladder prior to the test may cause confusing results due to the diuretic effect of the increased number of voidings.

The specimens were analyzed by the Benedict-Osterberg method, a method which we have found extremely simple and subject to very slight error in the hands of even relatively inexperienced operators.

With regard to this method, our experience has been that certain difficulties may be had in the preparation of suitable bone black. Bone black which liberates a great deal of  $H_2S$  and free S on treatment with HCl is very difficult to handle and does not effectively remove interfering substances.

Urine containing albumin causes precipitation on the addition of the picric acid, the precipitate redissolving on the addition of NaOH. We have found only slight variations in the sugar reading when the proteins were previously precipitated with phosphotungstic acid.

Benedict-Osterberg & Neuwirth<sup>2</sup> have shown that the quantity of sugar excreted is independent of volume. Our results amply confirm this view. We have used throughout our work the total sugar excreted in grams (volume  $\times$  per cent). Per cent seems to have very little significance.

The question of diet is an important one, though less so we must admit than we had believed at the beginning of the work. Two possibilities at least present themselves: (1) that we prescribe a standard diet for all; (2) that we allow the patient to eat the foods to which he is accustomed not only as to quality but also quantity.

So long as the diet is not excessive one way or the other the normal individual keeps well below the line of pathological wastage. Hence variations in diet are of little import. With the borderline diabetic this is a different matter.

In his case there are well defined peaks in the hourly sugar curve after food ingestion, the height of the peaks depending on the quality and quantity of food consumed. The following standard diet has been planned not to limit the patient, but to provide a good ration, quite suitable for the average adult doing a good day's work. In most borderline cases it is high in order that we may determine approximately what the patient can tolerate.

On the other hand certainly a great deal of information is obtained by allowing the patient to follow his own ideas as to diet. Probably for diagnostic purposes the unrestrained diet is the more desirable, but for compara-

tive purposes we have adopted the Standard Diet where it could be feasibly employed.

In the severe diabetics we have tried to employ a diet on which the patient was so-called "sugar free" to the ordinary Fehling and Benedict tests. We have indicated in each curve the exact diet, hence comparisons may readily be made.

STANDARD DIET									
	BREAKFAST					DINNER			
	C	P	F			C	P	F	
G. Fruit	5.0	2.0	0.9	Butter					16.2
Sh. Wheat	23.6	3.1	0.4						
Cream	4.6	2.6	19.0	Salad	{ Orange	10.0			
Toast	40.4	6.8	1.2		{ Apple	7.1	0.4	0.2	
Sugar	7.0				{ Celery	1.5	0.6	0.4	
Butter				Caulif. or 5%					
			16.2	Vegetable		1.5	0.6	0.4	
				Baked apple		14.2	0.8	0.4	
				Bread		20.2	3.4	0.6	
				Milk		5.0	3.3	4.0	
	81	13	37						
						70	9	22	
				SUPPER					
				C	P	F			
				Veg. soup	6.0	2.0	0.9		
				Potato	20.8	2.4	0.1		
				Milk	5.0	3.3	4.0		
				Steak		21.9	20.4		
				Ice cream					
				50 gms.	36.2	10.6	47.3		
				Butter			8.1		
					68	40	81		
Total C. = 219. P. = 62. F. = 140.									
Total glucose according to Woodyatt's method of estimation = 269.									

#### NORMAL SUGAR EXCRETION

In order to evaluate pathological sugar curves, we must obviously understand the normal. Our first work was carried out with the intention of determining a standard curve of sugar excretion. Four subjects were selected, all in excellent health and performing the same type of work. Specimens were voided at 7:00 A.M., 12:00 M., 5:30 P.M. and 10:30 P.M. The following table gives the results of 216 analyses.

TABLE I  
STATISTICAL TABLE OF NORMAL URINE SUGARS

SUBJECT	AVERAGE TOTAL SUGAR				TOTAL
	7:00 A. M.	12. M.	5:30 P. M.	10:30 P. M.	
P	.252	.189	.207	.221	.869
R	.328	.233	.293	.339	1.193
SW	.339	.218	.377	.388	1.322
Sh	.339	.234	.237	.240	1.050
Average hourly	.039	.043	.050	.054	

The period from 10:30 P.M. to 7:00 A.M. exhibits the lowest level of excretion. From 7:00 A.M. the level of excretion slowly rises. It must be



TABLE II  
 HOURLY URINE SUGAR IN NORMAL INDIVIDUALS

SUBJECT	TIME	VOLUME	TOTAL SUGAR IN GRAMS	DIET	SUBJECT	TIME	VOLUME	TOTAL SUGAR IN GRAMS	DIET
No. 1. A.J.S.	12 hr.	425	.857	Stand.	No. 3. E.S.	12 hr.	1000	.450	Stand.
	8 A. M.	55	.047			8 A. M.	36	.018	
	9 "	36	.039			9 "	27	.011	
	10 "	52	.048			10 "	23	.009	
	11 "	41	.037			11 "	31	.012	
	12 M.	55	.031			12 M.	28	.007	
	1 P. M.	90	.051			1 "	21	.015	
	2 "	100	.080			2 "	15	.009	
	3 "	40	.040			3 "	33	.026	
	4 "	32	.029			4 "	24	.016	
	5 "	26	.024			5 "	31	.019	
	6 "	60	.043			6 "	21	.009	
	7 "	40	.040			7 "	20	.009	
No. 2. E.B.	7 A. M.	53	.031	Stand.	No. 4. I.H.P.	8 hr.	325	.325	Mixed
	8 "	20	.020			8 A. M.	50	.034	
	9 "	45	.047			8:45 "	50	.025	
	10 "	70	.052			10 "	165	.082	
	11 "	83	.034			11 "	65	.055	
	12 M.	57	.023			12 M.	205	.059	
	1 P. M.	30	.033			1:30 P. M.	150	.046	
	2 "	62	.056			2:30 "	245	.076	
	3 "	55	.039			3:30 "	50	.030	
	4 "	70	.049			4:30 "	53	.042	
	5 "	62	.047			5:20 "	54	.036	
	6 "	47	.025			6:20 "	150	.040	
	12 hr.	717	.372			7:20 "	115	.040	
						8:20 "	90	.041	
						9:20 "	40	.047	
						10:20 "	50	.058	
						5 A. M.	240	.218	
						7:20 "	100	.075	
E.B.	7 A. M.	50	.047	Stand.	I.H.P.	8:20 A. M.	90	.029	Mixed
	8 "	45	.052			9:20 "	340	.034	
	9:10 "	85	.063			10:20 "	210	.067	
	10:15 "	265	.073			11:20 "	195	.019	
	11 "	90	.028			1:20 "	200	.020	
	12 M.	145	.062			12:10 P. M.	155	.054	
	1 P. M.	65	.041			2:20 "	75	.054	
	2 "	75	.058			3:20 "	95	.059	
	3:20 "	110	.076			4:20 "	115	.028	
	4 "	65	.039			5:20 "	230	.049	
	5 "	120	.077			6:50 "	140	.035	
	6 "	55	.045			8:00 "	60	.023	
	12 hr.	1100	.479			9 "	75	.038	
E.B.	7 A. M.	53	.032	Stand.		10 "	55	.040	
	8 "	65	.046			6:00 A. M.	285	.185	
	9 "	90	.044			8 "	190	.039	
	10 "	65	.033		No. 5. Reid	8 A. M.	35	.026	Stand.
	11 "	70	.039			9 "	232	.058	
	12 M.	45	.031			10 "	80	.040	
	1 P. M.	120	.067			12 M.	45	.031	
	2 "	70	.036			1 P. M.	48	.028	
	3 "	68	.043			11 "	48	.021	
	4 "	50	.033			2 "	140	.068	
	5 "	52	.033			3 "	98	.064	
	6 "	30	.024			4 "	36	.027	
	12 hr.	1110	.555			5 "	42	.036	
						6 "	34	.027	
						7 "	32	.021	
						12 hr.	600	.366	

SUBJECT	TIME	VOLUME	TOTAL SUGAR IN GRAMS	DIET	SUBJECT	TIME	VOLUME	TOTAL SUGAR IN GRAMS	DIET
No. 6. C.H.	12 hr.	720	.248	Stand.	No. 8. R.	12 hr.	260	.153	Stand.
	8 A. M.	70	.043			8 A. M.	36	.018	
	9:20 "	38	.023			9 "	26	.019	
	10:10 "	90	.032			10 "	47	.030	
	11:15 "	170	.059			11 "	70	.029	
	12 M.	50	.016			12 M.	31	.017	
	1 P. M.	50	.027			1 P. M.	36	.018	
	2:15 "	160	.062			2 "	..	....	
	3 "	58	.037			3 "	63	.063	
	4 "	260	.078			4 "	25	.018	
	5 "	240	.072			5 "	39	.024	
	7:15 "	110	.040			6 "	40	.021	
	8:30 "	85	.041			7 "	32	.020	
No. 7. J.M.	8 A. M.	35	.020		No. 9. J.W.	9 A. M.	50	.040	Mixed
	9 "	30	.030			10 "	50	.040	
	10 "	53	.039			11 "	45	.038	
	11 "	115	.049			12 M.	35	.026	
	12 M.	43	.023			1 P. M.	70	.042	
	1:15 P. M.	47	.033			2 "	45	.041	
	2:30 "	35	.044			3 "	30	.033	
	3:30 "	30	.039			4 "	35	.049	
	4:30 "	40	.033			5 "	30	.039	
	5:15 "	22	.016			6 "	35	.033	
	6:15 "	55	.030			7 "	65	.033	
	7 "	38	.023			8 "	100	.058	
	12 hr.	890	.400						

remembered that this type of curve eliminates the hills and valleys, hence is not a true picture of the hourly excretion. The striking thing, however, is the remarkable constancy of sugar excretion.

A number of subjects were now selected as "normals" to be run on the basis of the standard diet and hourly specimens. Table II and Figs. 1 and 2 will give an example of the results. None of these subjects had a history of diabetes nor had sugar even been found in their urines. We may say that in a number of cases supposedly normal, the curve of sugar excretion was not normal according to our conception. These subjects on closer examination showed a borderline diabetes.

The results seem to indicate that in the truly normal individual the sugar curve does not at any time make an excursion above the 90 to 110 mg. level. Points above this level are regarded with suspicion unless subsequently shown to be due to some untoward cause, such, for example, as excessive nervousness, strain, etc. A comparison of the normal with the borderline diabetic curve, however, leaves little doubt as to the nature of the case.

A consideration of the curves will indicate the difficulty of generalizing. We may say that one hour after meals a distinct rise occurs followed by a fall to approximately the original level or below.

The quantity of food ingested does not appear to control this rise to any great extent. In fact the greatest rise appears to come after breakfast and not after supper as illustrated by the record of Mr. I. H. P.

The normal curve varies a great deal from individual to individual but

the one thing in common is that excursions of the curve above the 90 to 110 mg. level do not appear to occur. The cases of Mr. R. (Fig. 2, Table I) and Mr. S. (Table II) illustrate the low levels of normal sugar. Fig. 1 shows that in three successive days on the same diet the excretion from hour to hour is practically identical. However, other subjects apparently just as normal show wide variations from day to day; indeed the same individual (Mr. R.) showed considerable variation after a few days.

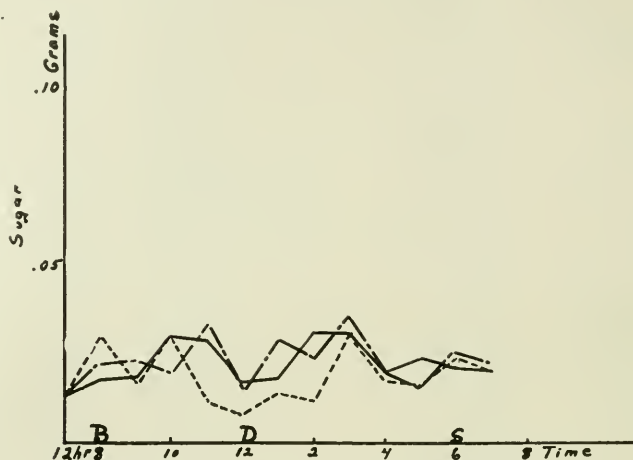


Fig. 1.—Normal hourly sugar curve (Mr. R.) on three successive days. B, breakfast; D, dinner; S, supper. Standard diet.

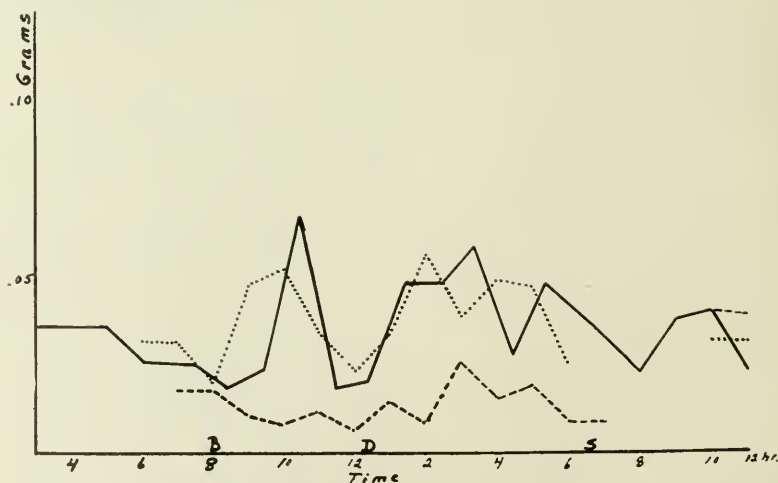


Fig. 2.—Hourly sugar curve of three normal individuals (Mr. B., Mr. S., and Mr. P.). B, breakfast; D, dinner; S, supper. Standard diet.

A great many factors must play a part in the excretion of "normal sugar"—for our purposes, however, we need only consider the causes which produce apparent anomalies in the curve. Certainly the most potent cause is defective sugar metabolism.

Our results would then seem to show (1), considered on a statistical basis, the curve of normal sugar excretion is remarkably constant but with

a slight upward trend from morning to evening, with a low level during sleep; (2), considered on the basis of hourly samples, the quantity of sugar excreted rises for one hour after meals, then sharply falls (the original on a lower level). The rise is in no way dependent upon the quantity or quality of food ingested. In general the greatest rise appears to follow breakfast; (3), the curve of our so-called normal cases does not exhibit excursions above the 90 to 110 mg. level; (4), great variations and remarkable constancies of sugar excretion may occur at different times in the same individual.

In all cases breakfast was eaten at approximately 7:15 to 7:30, dinner at 12 to 12:30 and supper 6:00 to 6:45.

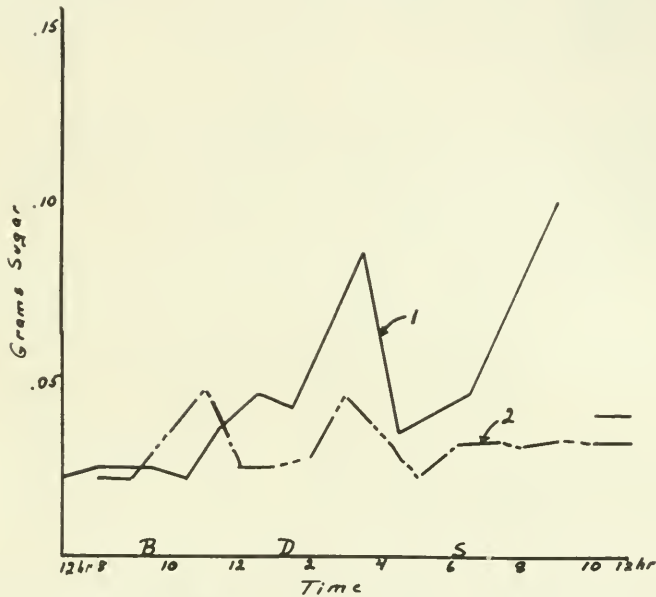


Fig. 3.—Hourly sugar curve of Mr. S., a very mild diabetic. Curve 1, test run on Sunday with no exercise—patient slightly nervous. Curve 2, taking exercise out of doors.

#### BORDERLINE AND SUSPECTED DIABETICS

The remarkable thing about this class of patients is their apparent ability to maintain a sugar-free urine except 2 to 4 hours a day. These so-called “breaks” generally occur  $1\frac{1}{2}$  hours or longer after meals and may last one to two hours. Following such a break a precipitous fall supervenes which may last three to four hours, again followed by another break. We have considered any excursion above the 90 to 110 mg. level as a “break.” However, as Figs. 3 and 4, Tables III and IV, will show, one is seldom in doubt as to whether or not a break has occurred.

#### CASE RECORDS

CASE 10, T. S.:—Sugar first noticed in August of 1921. The sugar was not detected until the third examination. The week before the test he had been under excessive nervous strain in court. Weight fluctuations have been very marked. Since the appearance of sugar he has lost 16 lbs, but appears to be fairly stable at the date of the tests.



TABLE OF HISTORIES III

SUBJECT	SEX	AGE	HEIGHT	WEIGHT POUNDS	VASCULAR CONDITION	KIDNEYS	OCCUPATION
No. 10. T. S.	Male	36	6'	184	Good	Good	Lawyer
No. 11. H. E.	Female	52	5' 5"	139	Good	Good	Head bookkeeper
No. 12. E. B.	Female	77	5' 6"	140	Fair	Alb.	Housewife
No. 13. H.	Male	29	5' 9"	125	Vascular trouble	Good	Machinist
No. 14. J.	Male	26	6' ½"	131	Palpitation	Good	Telegraph operator
No. 15. McN.	Male	58	5' 10"	167	Good	Good	Manufacturer
No. 16. Rev. W.	Male	43			Slight sclerosis	Good	Minister
No. 17. Dr. B.	Male	36	5' 9¾"	204	Good	Good	Doctor
No. 18. So.	Male	38	5' 8½"	152	Good	Good	Doctor
No. 19. Co.	Male	70	4' 5"	133	Sclerotic	Fair	Retired merchant

Fig. 3. Curve 1. 4/15/23 Breakfast C=62, P=44, F=42, Lunch C=2, P=13, F=25, Supper C=30, P=31, F=33. Total C=94, P=88, F=110. T. G.=156.

The test was made on Sunday, following a three day trip. T. S. sometimes showed sugar after such a trip but found no sugar on Saturday night. He did not take his accustomed exercise on Sunday and became slightly nervous toward evening.

Curve 2. 4/22/23 Breakfast C=81, P=13, F=37, Lunch C=70, P=9, F=22, Supper C=68, P=40, F=81, Total C=219, P=62, F=150. T. G.=270. Test made on Sunday. Patient worked on his car all day out of doors and felt in excellent condition.

Table IV. 5/2/23 Breakfast C=121, P=37, F=65, Lunch C=100, P=29, F=61, Supper C=58, P=45, F=56, Total C=279, P=111, F=182, T. G.=361.

The test was made during an average day's work at his office. No special worry was involved.

It is interesting in this case to note that T. S. exhibited a break on the day on which the total glucose content of the meals was least (T. G.=156). While doing his regular day's work at the office even though the glucose content of the diet was three times that of the diet on which he "broke" the curve appears quite normal.

CASE 11, H. E.:—Sugar first noticed October 1922. No sugar found 3 years before when a tonsillectomy was performed. Patient was losing weight rapidly up to November, 1922, when she was put to bed for a general rest to clear her of sugar. During 1921-1922 she had noticed some signs of polyphagia, and polyuria. She had been under excessive mental strain shortly before sugar was found. Her mother and father both had diabetes, but none has been found in her brother so far as she knew. The patient was able to do a normal days work and apparently enjoyed reasonably good health.

Fig. 4. Curve 1. 4/22/23. The Standard Diet was used, the test was made on Sunday, thus giving the patient an easy, restful day. This diet is not above that to which the patient was accustomed.

Table IV. B. 5/3/23. The Standard Diet was again followed but the test was run during regular office hours. Consideration of these curves leaves little doubt as to the existence of disordered sugar metabolism. Surely it is such cases as these that we must stop from their downhill progress.

CASE 12, E. B.:—Sugar was first noted 20 years before test. At the time none of the cardinal symptoms of diabetes were exhibited. She continued to show small amounts of sugar until a gangrenous toe of eight years' standing was amputated. About one year after this her leg was accidentally hurt and up to date, three years in all, has not healed. Her weight has been fairly constant and other than the wound on her leg, she appears in excellent health. We, of course, suspected defective sugar metabolism as the cause of the nonhealing of the wound. Aside from a slightly erratic curve we have not been able to detect any diabetes.

Table IV. 4/18/23. Standard Diet. Patient spent a quiet day at home. Some albumin was found.

Table IV. 5/6/23. The test was run on Sunday using the Standard Diet. The day was quiet and uneventful.

CASE 13, Mr. H.:—So far as the patient knew this was the first time sugar had been found. He entered the hospital to be under observation for appendicitis. The only history of diabetes was the death of a great aunt from that disorder.

Table IV. 4/4/23. Breakfast, C=40, P=8, F=30, Lunch, C=60, P=12, F=36, Supper, C=183, P=50, F=116, Total C=283, P=70, F=183, T. G.=342. The case was ambulatory but most of the test day was spent in bed.

CASE 14, Mr. J.:—No sugar had been found previous to this test. The family history was negative so far as diabetes was concerned. He entered the hospital for nervousness. Polyuria was rather marked but only a slight polyphagia.

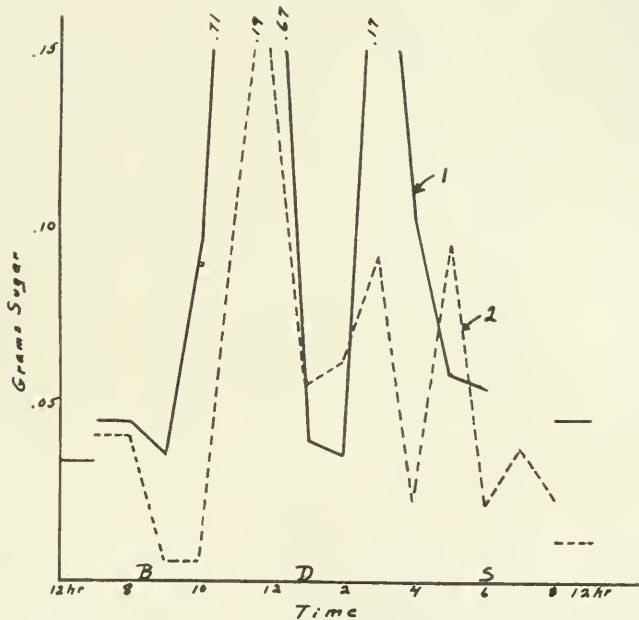


FIG. 4.—Hourly sugar curve of two suspected diabetics on standard diets. Curve 1, Miss E., and Curve 2, Rev. W.

Table IV. 3/4/23. Breakfast, C=63, P=11, F=31, Lunch, C=81, P=13, F=7, Supper, C=81, P=13, F=7, Total C=225, P=47, F=45, T. G.=257.

CASE 15, Mr. McN.:—A slight amount of sugar was first noted six weeks before the test. The family history was negative for diabetes. The patient appeared in excellent physical condition. Fasting blood sugar=.089%.

Table IV. 4/6/23. The Standard Diet was used.

CASE 16.—Rev. W. Sugar first found when the patient was a child. Had been again found three days before our test, in one specimen only (after noon meal), examination showed a badly deflected septum, chronic congestion and poor drainage of the sinuses, irritable colon, nervousness, abdominal discomfort and headaches. Fasting blood sugar=.087% while 11:00 A. M. Blood sugar=.167%.

Fig. 4, 4/9/23. The Standard Diet was used during the test. Total C=219, P=62, F=140, T. G.=269.

CASE 17.—B. Apparently has shown small amounts of sugar intermittently for two years. Basal metabolism=20%.

TABLE IV  
 BORDERLINE OR SUSPECTED DIABETICS

CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS	CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS
No. 10. T.S.	5/2/23			No. 13. H.	4/4/23		
	7 A. M.	1160	.243		9 A. M.	60	.052
	8 "	85	.030		10 "	170	.040
	9 "	144	.049		11 "	190	.024
	10 "	71	.045		12 M.	180	.019
	11 "	45	.032		1 P. M.	80	.037
	12 M.	75	.048		2 "	380	.148
	1 P. M.	75	.060		3 "	200	.080
	2:35 "	100	.048		4 "	150	.015
	3:35 "	75	.034		5 "	280	.291
	4:35 "	60	.040		6 "	106	.108
	5:35 "	54	.030		7 "	55	.036
	6:35 "		.027		8 "	90	.168
	7:35 "		.030		9 "	60	.138
	12 hr.		.307		12 hr.	340	.102
No. 11. H.E.	5/3/23			No. 14. J.	3/4/23		
	12 hr.	500	1.06		9 A. M.	330	.003
	8 A. M.	325	.109		10 "	no voiding	
	9 "	285	.749		11 "	52	.023
	10 "	125	.832		12 M.	330	.004
	11 "	90	.642		1 P. M.	210	.037
	12 M.	70	.205		2 "	380	.034
	1 P. M.	45	.040		3 "	80	.014
	2 "	125	.056		4 "	250	.022
	3 "	110	.456		5 "	80	.025
	4 "	120	.999		6 "	60	.049
	5 "	65	.541		7 "	180	.221
	6 "	180	.257		8 "	450	.265
	12 hr.	800	.896		9 "	90	.036
					12 hr.	800	.416
No. 12. E.B.	4/18/23			No. 15. McN.	4/6/23		
	7 A. M.	57	.030		12 hr.	660	.217
	8 "	47	.042		8 A. M.	200	.128
	9 "	15	.014		9 "	115	.037
	10 "	40	.034		10 "	230	.055
	11 "	55	.049		11 "	200	.182
	12 M.	50	.042		12 M.	260	.189
	1 P. M.	30	.019		1 P. M.	65	.046
	2 "	25	.024		2 "	150	.033
	3 "	47	.049		3 "	150	.033
	4 "	45	.049		4 "	80	.030
	5 "	65	.057		5 "	290	.087
	6 "	67	.046		6 "	180	.088
	12 hr.	1255	.404		7 "	60	.010
					8 "	110	.063
					12 hr.	500	.115
No. 12. E.B.	5/6/23			No. 17. B.	4/18/23		
	8 A. M.	120	.059		10 A. M.	100	.088
	9 "	47	.048		11 "	38	.030
	10 "	15	.015		12 M.	42	.031
	11 "	45	.052		1 P. M.	25	.021
	12 M.	55	.036		2 "	55	.052
	1 P. M.	55	.067		3 "		
	2 "	25	.020		4 "	100	.097
	3 "	30	.029		5 "	90	.066
	4 "	60	.042		6 "	25	.017
	5 "	55	.036		7 "	40	.036
	6 "	87	.050		8 "	50	.050
	7 "	50	.036		12 hr.	450	.351
	12 hr.	1130	.588				

TABLE IV—CONT'D.  
BORDERLINE OR SUSPECTED DIABETICS

CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS	CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS
No. 17. B.	4/24/23			No. 18. Sp.	4/15/23		
	10 A. M.	65	.079		8 A. M.	25	.020
	11 "	65	.057		9 "	35	.059
	12 M.	50	.048		10 "	35	.049
	1 P. M.	30	.029		11 "	30	.037
	2 "	30	.027		12 M.	25	.031
	3 "	52	.042		1 P. M.	25	.035
	4 "	60	.048		2 "	35	.159
	5 "	65	.042		3 "	35	.117
	6 "	55	.048		4 "	20	.034
	7 "	38	.043		5 "	40	.056
	12 hr.	475	.365		6 "	25	.031
No. 18. Sp.	2/6/23				7 "	30	.060
	11 A. M.	160	.209		8 "	55	.093
	12 M.	120	.051		9 "	45	.037
	1 P. M.	125	.042		10 "	120	.085
	2 "	120	.033		7 A. M.	890	.400
	3 "	140	.109	No. 19. Co.	9 A. M.	25	.025
	4 "	80	.066		10 "	200	.066
	5 "	150	.076		11 "	350	.129
	6 "	150	.046		12 M.	210	.065
	7 "	160	.009		1 P. M.	180	.075
No. 18. Sp.	5/13/23				2 "	300	.117
	8 A. M.	45	.036		3 "	100	.041
	9 "	40	.055		4 "	150	.064
	10 "	25	.028		5 "	100	.041
	11 "	30	.035		6 "	150	.060
	12 M.	30	.033		7 "	75	.080
	1 P. M.	23	.031		12 hr.	525	.676
	2 "	48	.060				
	3 "	50	.051				
	4 "	50	.054				
	5 "	35	.032				
	6 "	35	.045				
	7 "	75	.089				
	12 hr.	890	.795				

Table IV. 4/18/23 and 4/24/23. The Standard Diet was used.

CASE 18. Sp. Sugar first noted in 1921 but disappeared until January, 1923. He had been frequently examined prior to 1921 but the results were negative. Patient does not know of any reason for the first appearance of sugar. At the time of the test No. 9 Sp. had a focal infection in a molar tooth and some infected tonsillar tissue. Fehling's solution tests had been negative and were negative at the time of the test. The results from the glucose tolerance test (85 grams glucose) were as follows:

	Blood Sugar	Urine sugar
Normal	.114	.02 %
1st hour	.142	3.0 %
2nd hour	.135	.8 %
3rd hour	.097	.01 %

Table IV. 2/6/23.

Test taken while patient was resting in the hospital. C=60, P=45, F=145, T. G.=100.

11:00 A. M. Blood sugar, 116%

Table IV. 5/13/23.

Test run on Sunday—some exercise and considerable rest. Standard Diet used.

Table IV. 5/15/23.



Patient operated in the morning and spent a very busy day at his office. The Standard Diet was again used.

CASE 19. Co.—A slight amount of sugar was first detected  $11\frac{1}{2}$  years before the test and has intermittently appeared since then. All blood sugars have been normal. The following results were obtained with the glucose tolerance test.

	Blood Sugar	Urine Sugar in grams
Normal	.088	.06
30 min. after glucose	.170	.17
$11\frac{1}{2}$ hrs.	.160	.18
3 hrs.	.073	.03

Diacetic and acetone free. Fasting blood sugar .104%.

At the present time the patient had some nitrogen retention (Blood Urea  $N_2=25$  mg. and uric acid 3.7 mg.). Parkinson's disease also was found.

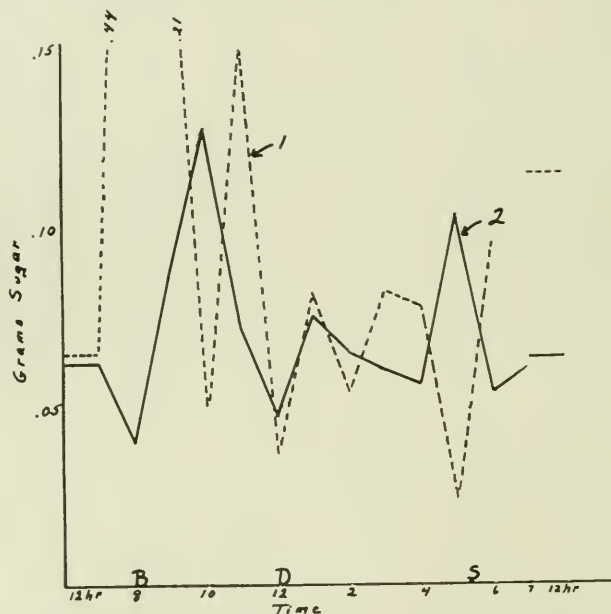


Fig. 5.—Hourly sugar curve of two very severe diabetics under insulin treatment. Curve 1, Mr. S. W., 6 units insulin at 8 a. m., 12 m., and 5 p. m. Curve 2, Mr. Wa., 1 units insulin at 8 a. m., 12 m., and 5 p. m.

#### BORDERLINE DIABETES UNDER INSULIN TREATMENT

It became of interest to determine what effect insulin would have on the small amounts of sugar intermittently thrown out by borderline diabetics. The following case will illustrate the effect.

CASE 30.—Mr. J. B. Sugar first noticed about 10 months before this test. Patient under treatment for bowel trouble when sugar was found. It appearance since then has been intermittent. No. 30 J. B. is Jewish and of a nervous disposition. His work required considerable mental strain and worry. Glucose tolerance test (85 grams glucose) showed apparently no abnormality whatever in the sugar metabolism.

	Blood Sugar	Total Grams Urine Sugar
Normal Fasting	.83	.04
1st hour	.153	.10
2nd hour	.105	.09
3rd hour	.084	.04

Immediately after the test hourly specimens were voided and the normal urine sugar determined. Table VII 5/10/23 presents the results with the results from the following day on our Standard Diet.

## SEVERE DIABETICS UNDER INSULIN TREATMENT

The following group of patients comprise the group known as severe diabetics. In practically all cases the patients had been desugarized by the insulin treatment.

TABLE OF HISTORIES OF DIABETICS UNDER INSULIN TREATMENT (SEE TABLE V)

NO.	SUBJECT	SEX	AGE	WEIGHT LBS.	HEIGHT	VASCULAR	KIDNEYS	OCCUPATION
No. 20	Swa	Male	42	92	5' 11"	good	fair	Manager
No. 21	Watj.	Male	28	129	5' 8"	good	good	Coal Miner
No. 22	Wes.	Male	14	72	5' 2"	good	good	School Boy
No. 23	H	Male	17	103	5' 6"	good	Alb.	Student
No. 24	E	Male	24	110	5' 6 $\frac{3}{4}$ "	good	good	Student
No. 25	Swin.	Male		187	5' 9"	good	Alb.	
No. 26	Crawf.	Male	16			good		School Boy

CASE 20.—Swa. A very severe diabetic of 3 years' standing. Blood sugar at 11 A. M. one day of test=212%. Fig. 5 No. 1. Total C=65, P=40, F=150, T. G.=103. The diet was equally distributed throughout the three meals. Six units of insulin were given 15 minutes before each meal.

CASE 21.—Wa. This is a case of fair severity with onset following an attack of 'flu.' Fig. 5 No. 2. Total C=70, P=45, F=170, T. G.=113 equally distributed throughout the three meals. Four units of insulin were given 15 minutes before each meal.

CASE 22.—Mr. W. Twelve days before the test the patient was in coma, with blood sugar of .666%, on insulin he became sugar-free in six days. Twelve days after his coma the test was run. Blood sugar on test day=100%. Fig. 6 No. 2, 5/3/23. Total C=75, P=35, F=140, T. G.=109 equally distributed throughout 3 meals. Two units insulin at 8 A. M. and 2 units at 5:00 P. M.

CASE 23.—Mr. H. This is a case of severe diabetes sugar-free on insulin. 11:00 A. M. blood sugar equalled .085% on the day of the first test.

Fig. 6 No. 1. Total C=80, P=45, F=185, T. G.=125 equally distributed throughout 3 meals. Five units of insulin were given at 8:00 A. M.

Table V. 4/12/23. Diet as in Fig. 5 No. 1 but 3 units of insulin were given at 8 A. M. and 3 units at 5 P. M.

CASE 24.—Mr. E. A case exhibiting all the cardinal symptoms of severe diabetes of four years' standing. Table V. 3/5/23. Total C=70, P=45, F=160, T. G.=122. Twelve units of insulin given at 8 A. M., twelve units at 12 M. and twelve units at 5 P. M. Table V. 3/6/23. Diet and insulin same as in curve A.

CASE 25.—Mr. Swin. Sugar first noticed in October of 1922. Cardinal symptoms of diabetes mild, but the patient was hard to desugarize. Table V. 3/2/23. Total C=65, P=40, F=160, T. G.=104. The patient had breakfast but no lunch. Blood sugars at 11 A. M.=200%, 12 M.=265% 1 P. M.=235%, 2 P. M.=250%, 3 P. M.=247%, 5 P. M.=220% Thirty units insulin were given by mouth at 11 A. M. with 6 oz. ice water. In this form the insulin is obviously ineffective. Two units insulin were given subcutaneously at 5 P. M. C=65, P=40, F=160 T. G.=104. Three units of insulin given at 8 A. M., 12 M. and 5 P. M. Total C=75, P=50, F=160, T. G.=122. Seven units of insulin were given at 8 A. M., 12 M. and 5 P. M.

TABLE V  
SEVERE DIABETICS UNDER INSULIN TREATMENT

CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS	CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS
No. 23. H.	4/12/23			No. 25. S.	3/2/23		
	9	20	.020		11	150	.610
	10	180	.041		12	120	.430
	11	220	.040		1	65	.180
	12	120	.037		2	75	.290
	1	220	.037		3	50	.110
	2	240	.053		4	50	.120
	3	160	.034		5	25	.040
	4	200	.050		6	30	.060
	5	100	.025		7	230	.250
No. 24. E.	6	100	.022		12 hr.	280	1.000
	7	140	.019	No. 25. S.	3/8/23		
	8	180	.029		12 hr.	600	1.134
	8	44	.070		8	150	.299
	9	33	.066		9	120	.114
	10	74	.042		10	180	.581
	11	119	.310		11	500	1.49
	12	89	.338		12	200	.624
	1	200	.156		1	280	.414
	2	130	.117		2	240	.480
	3	125	.100		3	320	.800
No. 24. E.	4	95	.098		4	150	.468
	5	100	.054		5	100	.143
	6	50	.025		6	120	.277
	7	100	.069	No. 25. S.	3/30/23		
	12 hr.	750	.405		12 hr.	700	.424
	10	220	.352		7	100	.050
	11	220	.242		8	100	.046
	12	50	.060		9		
	1	230	.078		10	500	.125
	2	280	.044		11	260	.084
	3	400	.120		12	400	.064
	4	240	.054		1	260	.006
	5	110	.041		2	320	.114
No. 24. E.	6	900	.050		3	120	.055
	7	280	.058		4	100	.047
	12 hr.	600	.240		5	120	.064
					6	80	.038

## GLUCOSE TOLERANCE TEST

	BLOOD SUGAR	TOTAL URINE SUGAR GRAMS
Normal	.080%	.070
1st hour after glucose	.172%	.056
2nd hour	.143%	.090
3rd hour	.090%	.041
4th hour	.082%	.022
5th hour	.078%	.022

## RENAL DIABETES

CASE 26.—Cr. First showed sugar at two years of age and has maintained a persistent glycosuria with a constantly normal blood sugar. The glucose tolerance test was not abnormal.

This case has shown some acetone and acetoacetic acid thus pointing to some complicating pancreatic diabetes. The insulin treatment seems to have a certain amount of value in this case.

Table VI. 4/18/23. Total C=65, P=40, F=165, T. G.=104. Samples were taken at two hourly intervals—for comparison with the other data, they must be calculated to an hourly

basis. This curve has been repeated twice on the same diet with results that are practically identical. Table VI. 5/1/23. Total C=85, P=50, F=190, T. G.=128. These units of insulin at 8 A. M. and 5 P. M. were given. Samples in this case were collected hourly. Table VI. 2/26/23. Total C=65, P=40, F=165, T. G.=104.

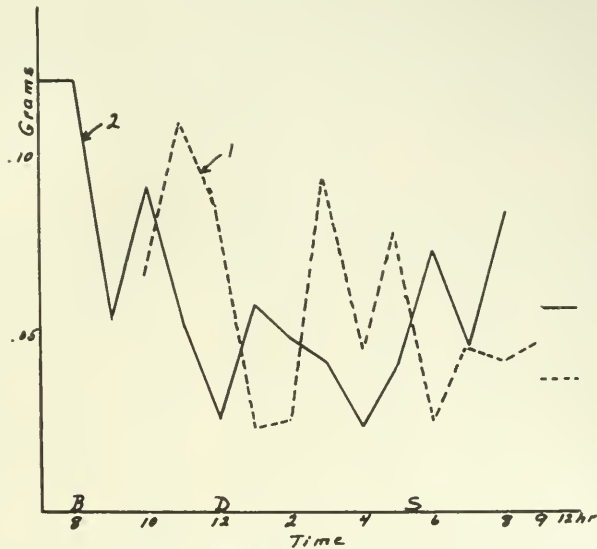


Fig. 6.—Hourly sugar curve of two diabetics undergoing insulin treatment. Curve 1, Mr. H., 5 units insulin at 8 a. m. Curve 2, Mr. W., 2 units insulin at 8 a. m., and 5 p. m.

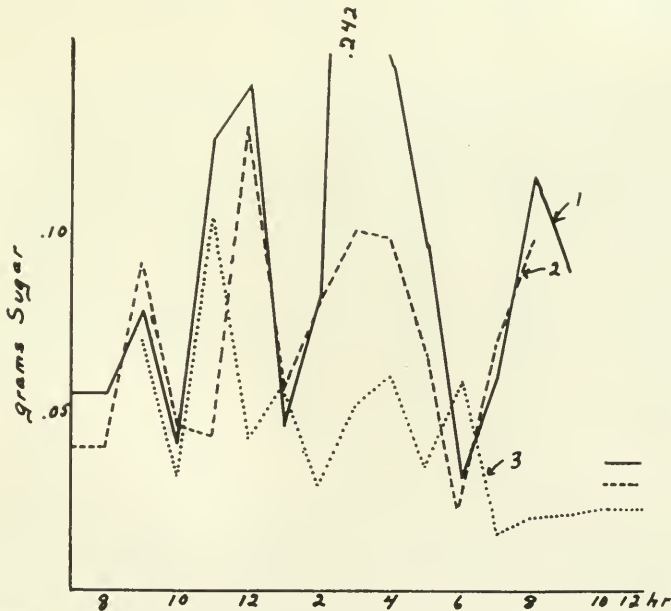


Fig. 7.—Mr. J. B. Borderline diabetes under insulin treatment. Curve 1, control day on standard diet. Curve 2, standard diet with three units of insulin at 10 a. m., and 5:30 p. m. Curve 3, standard diet with two units of insulin at 8:30 a. m., 12 m., and 5:30 p. m. Meals were served at 8 a. m., 12:30 p. m., and 5:45 p. m.

It is very striking that shortly after a so-called "normal" glucose tolerance test the patient exhibited three excessive "breaks." A week after this test No. 30, J. B., again returned to the hospital. Fig. 7, Curve No. 1, pre-



sents the first control day. Curve No. 2 presents the following day with 3 units insulin at 10 A.M. and 3 units at 6 P.M. Curve No. 3 presents the results of the next day with 2 units insulin at 8:30 A.M. and 2 units at 12 M., and 2 units at 5:30 P.M. The Standard Diet was used throughout.

It is a question for the future to decide whether insulin treatment would be an aid in preventing the downhill progress of such a mild glycosuria. Certainly it is a question of the utmost importance.

TABLE VI.  
RENAL GLYCOSURIA

CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS	CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS
No. 26, Cr.	4/18/23			No. 26, Cr.	5/1/23		
	9 A. M.	50	.185		7 A. M.	50	.217
	11 "	60	.190		8 "	60	.124
	1 P. M.	80	.260		9 "	60	.499
	3 "	80	.500		10 "	60	.461
	5 "	60	.190		11 "	40	.114
	7 "	80	.260		12:30 P. M.	80	.190
	12 hr.	900	1.278		2 "	80	.420
No. 26, Cr.	2/26/23				3 "	95	.286
	10 A. M.	60	1.2		4 "	60	.153
	12 M.	200	1.0		6 "	80	.144
	2 P. M.	220	2.4		12 hr.	950	1.43
	4 "	250	1.6				
	6 "	300	2.7				
	8 "	85	.7				
	12 hr.	460	3.5				

TABLE VII  
BORDERLINE DIABETIC AFTER TOLERANCE TEST

5/10/23	TIME	VOLUME	TOTAL SUGAR GRAMS	5/11/23	TIME	VOLUME	TOTAL SUGAR GRAMS
	9:50 A. M.	220	.098		9 hr.	610	.287
	10:50 "	150	.063		8 A. M.	30	.016
	11:50 "	325	.100		9 "	160	.064
	12:50 P. M.	425	.129		10 "	290	.088
	1:50 "	120	.050		11 "	245	.067
	2:50 "	200	.086		12 M.	425	.106
	3:50 "	400	.129		1 P. M.	250	.085
	4:50 "	425	.139		2 "	175	.068
	5:50 "	90	.038		3 "	75	.055
	6:50 "	100	.052		4 "	215	.086
	7:50 "	360	.450		5 "	150	.065
	8:50 "	120	.117		6 "	345	.143
					7 "	220	.101
					9 hr.	765	.382

Our experience with dogs has been that very considerably more insulin is required to bring the urine sugar below the "normal" level than is required to bring the excess sugar in a hyperglycemia down to this normal level. The diabetic is infinitely more sensitive to insulin than the normal individual.

#### HYPERTHYROID CASES

CASE 27.—Mr. Blen. Age sixty-nine, height 5' 8", weight 110 lbs. This case has had a colloid goiter for 30 to 35 years. Sugar was found in the urine about two months before this

TABLE VIII  
HYPERTHYROID CASES

CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS	CASE NO.	TIME	VOLUME	TOTAL SUGAR GRAMS
No. 27. Blair	12 hr.	1000	.062	No. 29 C. M	9 A. M.	50	.038
	9 A. M.	100	.057		11 " "	80	.060
	10 " "	30	.021		1 P. M.	120	.126
	11 " "	50	.040		3 " "	70	.144
	12 M.	75	.047		5 " "	100	.091
	1 P. M.	85	.042		7 " "	50	.116
	2 " "	75	.063		12 hr.	150	.151
	3 " "	65	.072		9 A. M.	20	.003
	4 " "	50	.038		11 " "	70	.074
	5 " "	120	.060		1 P. M.	50	.009
	6 " "	75	.042		3 " "	50	.007
	12 hr.	1250	.499		5 " "	100	.050
					7 " "	90	.051
No. 28. B.D.	12 hr.	560	.610		12 hr.	450	.024
	8 A. M.	80	.088		9 A. M.		
	9 " "	45	.049		11 " "	40	.031
	10 " "	50	.051		1 P. M.	80	.080
	11 " "	30	.033		4 " "	200	.156
	12 M.	45	.099		5 " "	70	.119
	1 P. M.	40	.066		7 " "	180	.417
	2 " "	40	.055		12 hr.	600	.300
	3 " "	30	.041		9 A. M.	50	.024
	4 " "	35	.040		1 P. M.	80	.115
	5 " "	45	.040		3 " "	50	.083
	6 " "	45	.035		5 " "	220	.261
	7 " "	50	.045		7 " "	80	.078
	12 hr.	760	.418		12 hr.	630	.598

test. Wassermann test was negative. The family history showed a high incidence of diabetes. Table VIII presents the results of one test. Total C=95, P=50, F=180, T. G.=142.

CASE 28.—Miss B. D. Age seventeen, height 5' 5", weight 105 lbs. Metabolism=41%. The patient showed very definite toxic goiter. Table VIII presents one test on the Standard Diet. Breakfast at 8 A. M., lunch at 12:30 and supper at 6 P. M. This case illustrates the lack of correlation of the "breaks" with the food ingestion.

CASE 29.—Mr. C. M. Age fifty-four, weight 48.5 kg., height 176 cm. A very definite case of hyperthyroidism. Basal metabolism=+55%. Table VIII gives the results of specimen 2. This case illustrates how variable such cases are with respect to sugar wastage.

## DISCUSSION

Kast, Croll and Myers<sup>3</sup> found in 12 normal individuals an average of .96 grams sugar excreted in 24 hours. Neuwirth<sup>4</sup> found .941 grams average in 26 normals while Folin and Berglund<sup>5</sup> found 1.203 grams in one normal. Our results show an average of 1.015 grams for 10 normals, 72 analyses being involved in the average.

Kast, Croll and Myers<sup>3</sup> in an extended investigation found only slight variations in the total sugar in the 24 hour specimen under greatly varying pathological conditions. Our own experience amply corroborates this work. We have therefore felt that little could be gained from a further pursuance of this line of study.

The curve of hourly sugar secretion suggested itself as a possible means of determining the state of sugar metabolism. As has been previously pointed out, peaks may occur in this curve which are entirely obliterated by compensatory valleys thus producing a combined specimen that is apparently not abnormal.

Empirically we have found that excursions of the hourly sugar curve above the 90 to 110 mg. level may be considered indication of abnormal carbohydrate metabolism. Individuals whom we have every reason to suspect as being entirely normal do not exhibit the so-called "breaks" or abnormal excursions of the sugar curve.

Consideration of the curves of the mild or suspected diabetics reveals how easily these cases might be overlooked by the single and 24 hour combined specimen tests. It would seem to be purely a matter of chance whether the examination was made during a break, in a valley or a composite of the two.

It is believed that the ability to detect latent, borderline or mild diabetes depends on the determination of the picture of hourly sugar secretion under the varying conditions of the patient's life. Certain cases (No. 1, Mr. S., as an example) so long as normal exercise was maintained kept his sugar curve normal in form but when subjected to excessive mental strain, general nervousness, etc.,—the result is a "break." Possibly the normal individual might do the same under very excessively abnormal conditions, but when one considers the form of the curve in connection with the clinical syndrome exhibited, little doubt is left as to the status of the case.

It would indeed be rash to claim that all hourly sugar wastage above the 90 to 110 mg. level proved the existence of diabetes mellitus. It is well known that pathological sugar excretions may be due to such conditions as hyperthyroidism, liver disorders, nervousness, etc. We have not in an examination of hyperthyroid cases found the type of curve characteristic of diabetes. In general it may be said that the borderline or mild diabetic exhibit "breaks" shortly after meals whereas, if a "break" occurs at all in hyperthyroid cases, it apparently has no relation to food ingestion.

Diet in the case of normal individuals so long as it is not excessively abnormal does not appear to play as important a rôle as might be expected. Certainly after food ingestion there is an increase in normal urine sugar secretion as Benedict, Osterberg and Neuwirth have shown. We have been able to find no proportionality between the height of the peak and the quality and quantity of food ingested. These peaks are generally followed by compensatory falls in this manner producing approximately a constant level of sugar excretion.

Diet in cases where diabetes is involved is a far more important matter. In such cases  $\frac{1}{4}$  to  $\frac{1}{2}$  hour after meals the sugar secretion rises sharply and the rise may show some proportionality to the total glucose content of the foodstuffs ingested.

A comparison of this test with the ordinary glucose tolerance test may bring certain points of interest.

Gray<sup>6</sup> has shown in a statistical analysis of a very large quantity of published data that in patients clinically normal the fasting blood sugar may

vary from .04 to .16 per cent, the average being .09 to .10 per cent. After the ingestion of 100 grams of glucose the peak may vary from .14 per cent to .28 per cent.

With regard to the quantity of glucose ingested for the tolerance test Gray states, "The highest peak with the smallest dose of glucose is as great as the average peak for the largest dose of glucose. So it seems fairly reasonable to say that curves vary at least as much owing to individuals as owing to the size of the dose." Since Benedict, Osterberg and Neuwirth<sup>2</sup> have shown the marked influence of small amounts of food ingested along with the glucose it makes one feel, at least for borderline cases, the results of such a test would be decidedly doubtful.

Hamman and Hirschman<sup>7</sup> state that the duration of the glucose reaction is a more important index of the severity of the diabetes than is the height of the curve.

Gray further states, "Hence, it is conspicuous that a normal fasting blood sugar by no means excluded the presence of diabetes, and that the entire curve in a diabetic may be normal."

Our data amply confirms this statement. In all our borderline cases the fasting blood sugars and glucose tolerance tests have been entirely normal whereas the hourly sugar examination has demonstrated "break" after "break" in the sugar wastage.

Further we must recognize what an excessively abnormal condition is induced by the ingestion of 75 to 100 grams of glucose. Kawachi,<sup>8</sup> Martius,<sup>9</sup> Allen,<sup>10</sup> Ohler,<sup>11</sup> and Gray,<sup>6</sup> have sounded the warning against the danger to a subnormal pancreas of the use of the ordinary glucose tolerance test. Joslin and many others now use this test with great caution if at all.

We must also remember that the patient is subjected to a rather severe nervous strain, not only from the fact that the test is ordinarily conducted in the hospital, but also from the fear and pain involved in vein puncture for blood samples. The hourly urinary test may and should be conducted under the varying conditions of the patient's normal environment.

The hourly sugar curve gives us a complete picture of the progress of a patient so-called "sugar free." The curves presented will show how closely the sugar curve of a severe diabetic, properly treated with insulin, may be made to approximate that of the normal individual. Using the ordinary means of sugar determination, once the patient is desugarized, we must proceed in the dark. The normal sugar determination is applicable where the other tests are no longer available. The hourly curve offers the additional advantage of detecting "breaks" in the sugar metabolism. "These "breaks" may and should be covered by proper regulation of the insulin dosage and dietary therapeutics until by proper adjustment of dosage and diet, the hourly sugar curve becomes normal in form.

In the normal urine sugar we have not a picture of the momentary state of the sugar metabolism, but a 24 hour picture, after the great equalizer, the kidney, has played its part.

The dangers of insulin overdosage are well known to the users of insulin. The normal urine sugar method offers a means of determining the point where the danger zone of hypoglycemia is being approached. Our



results seem to demonstrate that a fall below the 30 mg. level in a *patient undergoing insulin treatment* should be regarded with considerable suspicion. As we have previously pointed out the amount of insulin required to reduce "normal urine sugar" to a dangerously low level is far in excess of that required to reduce pathological glucose wastage. This is indeed fortunate for were it not the case a patient would no sooner become sugar free than a hypoglycemic reaction would set in.

Hypoglycemic reactions are always marked by a powerful diuresis hence the total quantity of sugar excreted, unless this figure is calculated back to a normal basis for urine volume, may not indicate deficient blood sugar.

The Benedict, Osterberg test may be easily modified to give a method that could easily be run by patients. We have tried with success the following: Two c.c. urine plus 1 c.c. .6 per cent picric acid plus  $\frac{1}{2}$  c.c. .5 per cent NaOH plus 5 drops 50 per cent acetone mixed in an ordinary 20 c.c. test tube. The contents boiled 15 seconds and filled to a file scratch at 10 c.c. These tubes are then compared with permanent picramic acid standards.

Another phase and possibly the most important one of the normal urine sugar question is the plan of treatment to be followed with these borderline cases. It is believed that the method here described will detect a great many cases formerly overlooked. In one case, No. 30, J. B., who had a normal fasting blood sugar and glucose tolerance test, but exhibited pathological breaks according to our conception, we have given insulin. Fig. 7 shows quite definitely that the hourly sugar curve quickly became normal. Whether such patients had better be kept on a reduced diet without insulin or with insulin is a question for the future to decide.

In conclusion I wish to express my indebtedness to Dr. G. H. A. Clowes for advice and suggestions in the conduct of the work, to Mr. W. O. Rader for assistance in carrying out the laboratory work, to Drs. J. A. MacDonald, Louis Burekhardt, C. L. Rudesill and J. H. Warvel for providing clinical material, and to Miss Michael and Miss Thompson for cooperation on the clinical side.

#### SUMMARY

1. The hourly urine sugar curve has been determined in (1) Normal individuals; (2) Borderline diabetics; (3) Severe diabetics under insulin treatment; (4) Renal diabetes; (5) Borderline glycosuria under insulin treatment; (6) Hyperthyroid cases.

2. The use of insulin in borderline glycosuria has been briefly considered.

3. A standard technic has been described for the determination of hourly sugar curve.

4. It is believed that an examination of the hourly sugar curve will demonstrate cases of mildly pathological sugar metabolism that would be overlooked by the ordinary tests for such conditions.

5. A comparison of this test with the glucose tolerance test we believe demonstrates that in many ways it is very superior.

6. The method on the one hand detects abnormally high sugar wastage

and on the other warns of dangerously low sugar secretion, hence offers an almost ideal method for the control of cases of diabetes under insulin treatment.

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## THE SIGNIFICANCE OF NEGATIVE RESULTS IN BLOOD CULTURES\*

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## THE SIGNIFICANCE OF NEGATIVE RESULTS IN BLOOD CULTURES

THE literature contains many reports of the bacteriologic examinations of the blood, and various infections have been studied in this way. Following is a list of the more important diseases in which positive results have been obtained: typhoid and paratyphoid fevers, pneumonia, infectious endocarditis, erysipelas, postpartum infections, cholecystitis, pyelitis, pyosalpinx, otitic infections, local infections and abscesses in various parts of the body, osteomyelitis, glanders, general cryptogenetic infections, certain cases of diphtheria, and in many of the obscure febrile cases so frequently seen in our hospital wards.

The usefulness of the bacteriologic examination of the blood in many infectious diseases is self-evident. Probably no other single method of diagnosis has yet been devised that furnishes of itself so definite and clear diagnosis as does this method. Its usefulness is, to be sure, limited to certain kinds of cases, but these cases are numerous and are often extremely difficult or impossible to diagnose by any other means.

While the literature upon the significance of positive blood cultures is voluminous, it contains no experimental work upon the possible meaning of negative blood cultures that meets modern bacteriologic requirements. The relatively large number of cases reported as giving a negative blood culture have not yet been explained by experimental evidence. Table I shows some of the reported cases which illustrate the importance of positive findings as well as the importance of knowing the meaning of the large percentage of negative findings.

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Table I shows that of a total of 2,284 cases, 1,514 cases (or 66.29 per cent) were positive, while 770 cases (or 33.72 per cent) were negative. The examinations were made at various stages of the diseases and by different methods. What does this large percentage of negative results mean? To answer this question, several factors must be taken in consideration. A few of the more important ones are here considered:

TABLE I  
SOME REPORTED CASES

NO.	AUTHORS	ORGANISM	NUMBER OF CASES	POSITIVE	NEGATIVE
1	Auerbach and Unger	B. Typhosus	10	7	3
2	Bosquet	" "	83	83	0
3	Berri	" "	13	7	6
4	Brion and Kayser	" "	233	153	80
5	Cole	" "	15	11	4
6	Courmont and Lesieur	" "	37	37	0
7	Castellani	" "	14	11	3
8	Coleman and Buxton	" "	123	81	42
9	Duffy	" "	88	62	26
10	Epstein	" "	230	170	60
11	Eppenstein and Korte	" "	6	5	1
12	Fox	" "	14	1	13
13	Fornet	" "	19	14	5
14	Hewlett	" "	25	21	4
15	Hirsh	" "	97	75	22
16	Harrison	" "	8	5	3
17	Janesco	" "	19	8	11
18	Jochmann	" "	30	25	5
19	Kerr and Harris	" "	56	31	25
20	Kuchmau	" "	21	9	12
21	Korte and Sternberg	" "	22	22	0
22	Kayser	" "	75	50	25
23	Loiseleur	" "	65	63	2
24	Memmi	" "	30	18	12
25	Perquis	" "	40	30	10
26	Ruediger	" "	32	20	12
27	Rolly	" "	50	43	7
28	Richard	" "	50	44	6
29	Rusta	" "	12	12	0
30	Schottmaeller	" "	119	98	21
31	Todd	" "	23	17	6
32	Trappe	" "	38	25	13
33	Widal	" "	25	17	8
34	Warfield	" "	48	33	15
35	Dul and Wright	Streptococci	57	16	41
36	Libman and Celler	" "	30	23	7
37	Spassokukotsky	" "	81	52	29
38	Raffaelli	Meningococci	22	20	2
39	Ryttenberg	Various bacteria	324	87	237
			2,284	1,514	770

1. The bactericidal effect of the serum introduced into the culture media with each sample of blood.

2. Number of organisms in infected blood in various pathologic conditions.

3. The viability of different bacteria.

4. Infection not due to bacteria.

*Bactericidal Effect of Serum.*—Much of the difficulty hitherto experienced

in cultivating the organisms found in the blood has been attributed to the bactericidal power of the blood. To overcome this Castellani<sup>1</sup> used large quantities of bouillon, and obtained positive results in 78 per cent of the cases of typhoid fever; Schottmüller,<sup>2</sup> however, used agar, the blood being present in the proportion of 1 to 3 in the medium and obtained positive results in 81 per cent of the cases in typhoid fever. Numerous other methods

NO.	QUANTITY OF BLOOD IN EACH PLATE	NUMBER OF COLONIES PER PLATE AT DIFFERENT INTERVALS						
		0 MIN.	15 MIN.	30 MIN.	1 HR.	2 HRS.	3 HRS.	4 HRS.
1	0.10 c.c.	39	35	107	138	115	195	324
2	0.09 "	12	11	69	125	108	150	208
3	0.08 "	18	10	52	104	97	133	276
4	0.07 "	14	8	15	118	84	101	312
5	0.06 "	4	7	14	102	79	86	121
6	0.05 "	0	3	15	88	63	69	100
7	0.04 "	6	5	8	61	31	57	69
8	0.03 "	3	0	14	59	45	49	52
9	0.02 "	0	1	4	36	13	12	45
10	0.01 "	0	0	0	27	16	14	18

have been employed by different investigators. Lemiere<sup>3</sup> was successful with cultures made from defibrinated blood of a typhoid fever patient. Muller and Graef<sup>4</sup> obtained growth of the *B. typhosus* in the fibrin net-work of clotted blood. Klodnitsky<sup>5</sup> succeeded in growing the typhoid bacillus from blood laked in ordinary sterile water. Epstein<sup>6</sup> found multiplication of the typhoid *B.* in oxalated blood in twenty-three out of twenty-five cultures without the addition of any nutrient material.

NO.	QUANTITY OF BLOOD IN EACH PLATE	NUMBER OF COLONIES PER PLATE AT DIFFERENT INTERVALS						
		0 MIN.	15 MIN.	30 MIN.	1 HR.	2 HRS.	3 HRS.	4 HRS.
1	0.10 c.c.	800	1,000	1,100	1,100	1,200	*	*
2	0.09 "	750	900	1,000	1,000	1,200	1,200	*
3	0.08 "	675	850	1,000	1,000	1,100	1,100	1,200
4	0.07 "	412	834	900	900	1,000	900	1,000
5	0.06 "	283	417	948	950	900	800	739
6	0.05 "	191	426	812	623	876	734	745
7	0.04 "	200	350	700	800	634	856	768
8	0.03 "	5	312	634	54	456	612	712
9	0.02 "	57	234	65	75	87	303	703
10	0.01 "	48	95	76	96	18	24	494

\*Too many for accurate count.

From these data it will be seen that the claim so frequently made that the failure of the bacteria to grow is wholly due to the continuation of the bactericidal action of the blood (in vitro) is not supported by experience, and has been considerably overrated. This view gains support from the fact that bacteria multiply in defibrinated, oxalated, laked and clotted blood without the addition of any nutrient medium.

The work of Eppenstein and Korte, and later of Epstein, as well as my own experience, indicate that the *B. typhosus*, *B. coli*, *B. mucosus capsulatus*, most streptococci, staphylococci, and pneumococci, *B. mallei*, resist the bactericidal action of the blood of the host and thus are not inhibited by any



bactericidal effect of the serum but tend to multiply. The method of adding a few drops of blood serum to broth for increasing viability and to improve growth of attenuated organisms is commonly employed. The following experiments will illustrate the above facts.

*Experiment 1.*—A loopful of a 20-hour broth culture of streptococcus hemolyticus was introduced into 10 c.c. sterile human blood. The following series of plates were poured at different intervals of time. During the four hours, the blood remained at room temperature (summer).

*Experiment 2.*—Six drops of sterile human blood serum was added to 10 c.c. sterile broth. This tube was inoculated with the organism used in the first experiment. After 20 hours' incubation, a loopful of this culture was introduced into 10 c.c. sterile human defibrinated blood. The following series of plates were poured at different intervals of time. During the four hours the blood remained at room temperature (summer).

It will be noticed that the number of organisms increased steadily the longer they were allowed to grow in the blood, and that they are more numerous in the table of Experiment 2 in which is the report of the culture containing six drops of serum. After the four hours, I incubated both tubes for twenty hours and then poured a series of ten plates with different quantities of blood from each tube. The number of organisms in each plate were so numerous that it was impossible to count them, proving that the streptococci grow well in human blood.

Taking the above facts into consideration, it becomes evident that in working with one or another method many of the real conditions underlying the success or the failure of blood-culture work have been overlooked.

*Number of Organisms.*—E. Libman<sup>7</sup> reported results obtained from studying 750 cases of bacteriemia by means of cultures. He found that "the number of organisms varied from one in fifteen c.c. up to 2676 to the c.c., or up to such a number that the colonies could not be counted." We also know that typhoid fever is a disease associated with relatively few organisms in the circulating blood, especially in the later period of the disease. Ryttenberg<sup>8</sup> found that twenty-six positive cultures representing one hundred and ninety c.c. of blood showed a total of one hundred and seventy-two colonies, an average of less than one colony per c.c. of blood. Of these twenty-six cultures the highest single count was about three colonies per c.c.; the lowest, one colony per ten c.c. of blood. We should also remember that some bacteriemias are caused by the liberation of bacterial emboli into the blood stream from some focus in the body, and that in these cases the blood may be periodically free from bacteria.

From the above data one sees that in a given sample of blood (usually not more than 10 c.c. and very frequently less than this quantity) containing very few organisms or a single organism, there is a large element of chance as to whether a given sample of blood will give a positive or negative culture.

To emphasize this element of chance in transient blood infections and bacteriemias associated with few organisms, I have performed the following experiment:

*Experiment 3.*—Six drops of sterile human blood serum was added to 10 c.c. sterile broth. This tube was inoculated with the same organism used in the first two experiments. After twenty hours' incubation, a loopful of this culture was introduced into 10 c.c. sterile human defibrinated blood. This tube is 1. A loopful of the blood of 1 was immediately introduced into another tube containing 10 c.c. human defibrinated blood. This tube is 2. The following series of plates were poured at different intervals of time.

NO.	QUANTITY OF BLOOD IN EACH PLATE	1		2	
		POURED INSTANTLY	POURED AFTER 24 HRS. INCUBATION	POURED INSTANTLY	POURED AFTER 24 HRS. INCUBATION
1	0.10 c.c.	276	*	3	*
2	0.09 "	112	*	0	*
3	0.08 "	161	*	0	*
4	0.07 "	204	*	0	1200
5	0.06 "	198	*	0	1000
6	0.05 "	107	*	0	*
7	0.04 "	91	1200	0	1000
8	0.03 "	98	1000	1	900
9	0.02 "	37	1200	0	800
10	0.01 "	12	1000	0	864

\*Too many for accurate count.

The number of sterile plates in the above experiment illustrates the fact that the element of chance plays a very important rôle in cases where the number of organisms in the blood is not abundant. In 2, when plated instantly, only two positive results were obtained, while when the same culture was allowed to incubate for twenty-four hours, all the plates showed numerous colonies. From the above experiment we may, therefore, conclude: first, that larger quantities of blood and smaller dilutions must be used, and, second, that the sample of blood should be first incubated for twenty-four hours before plates are poured or samples distributed to different medias.

*The Viability.*—This factor is not a negligible one. One must select the most favorable medium for the organism suspected. This can be accomplished best by carefully considering the history of the case.

Again the disease affecting the patient may not be of bacterial origin, but the symptoms presented may be similar to those produced by a bacterial septicemia. Malaria forms a typical example of this type of infection.

If blood cultures are taken more frequently when clearly indicated, and the suggestions here made observed, I feel that the diagnosis of infectious diseases would be materially aided, for quite frequently it is impossible to make one without it. Positive blood cultures establish a diagnosis as but few other single procedures do.

#### SUMMARY

The bactericidal influence of the blood drawn for blood cultures has been overestimated.

The element of chance in obtaining a positive or negative blood culture plays an important rôle.

Larger quantities of blood and lower dilutions should be used in order

to offset this element of chance. High dilutions of the sample of blood are not essential.

At times there may be an advantage in first incubating the sample of blood for twenty-four hours before it is distributed to different nutrient media or poured on to plates.

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### TOXICITY OF COCAIN AS INFLUENCED BY RATE OF ABSORPTION AND PRESENCE OF ADRENALIN\*

BY ELLISON L. ROSS, PH.D., M.D., CHICAGO, ILL.

THE toxic reaction sometimes occurring with the clinical use of cocain, is often explained by saying that for some reason the drug was absorbed with unusual rapidity. As far as we have been able to ascertain no accurate data has been collected on this point. Consequently, we thought it sufficiently important to determine, within certain limits, the variation in the minimal lethal dose with the rate of introduction into the circulation.

There is a difference of opinion expressed in the literature concerning the influence of adrenalin on the toxicity of cocain. Allen<sup>1</sup> in his book on anesthesia states that "Adrenalin may prove a distinct disadvantage after cocain." Allen quotes J. M. Berry as believing that adrenalin increases the toxicity of cocain. He also quotes Thriss, Miles, Braun and Petrow as saying that adrenalin is of benefit to the patient after a toxic dose of cocain.

Buxton<sup>2</sup> in his book on anesthetics says that "even minute doses of adrenalin are said to cause unpleasant effects, e.g., giddiness, fainting and collapse."

Mortimer<sup>3</sup> discussed the existing difference of opinion on the advantages and disadvantages of adrenalin with cocain. He argues against the free use of adrenalin with cocain because of the possibility of extreme cardiac dilatation making contraction impossible. This mechanical factor we found to be present to a marked degree, as was shown in the blood pressure tracing of a number of animals that had been given cocain and adrenalin.

Harris<sup>4</sup> reported a death resulting from an injection of  $\frac{1}{2}$  of a grain of cocain with 8 to 10 minims of adrenalin. Those discussing the paper were divided in opinion, some thought the death due to the adrenalin and one thought it due to the cocain.

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Hatcher and Wilbert<sup>5</sup> state that "artificial respiration and injection of epinephrin saved a certain number of animals after injection of doses of cocain that were 25 per cent larger than those subsequently required to cause death."

Sollmann<sup>6</sup> in referring to cocain says "the toxicity is greatly lessened by the addition of epinephrin."

Our work<sup>7</sup> strongly suggested to us that adrenalin increased the toxic effect of cocain. The great rise in arterial pressure and greater rise in intracranial venous pressure and strong stimulation of the vagus, produced by

TABLE I  
VARIATION OF TOXICITY DUE TO RATE OF ADMINISTRATION OF COCAIN

CAT	RATE OF INJECTIONS GM. PER MINUTE	LETHAL DOSE COCAIN—GM. PER KILO.	
		RESPIRATION STOP	HEART STOP
27	.0115	.0114	.0208
28	.0115	.0157	.0249
29	.0115	.0114	.0181
1	.0057	.0144	.0212
2	.0057	.0168	.0190
3	.0057	.0141	.0181
32	.0028	.0195	.0214
33	.0028	.0225	.0263
35	.0028	.0196	.0215
40	.0230	.0156	.0422
41	.0230	.0165	.0387
42	.0230	.0128	.0267

TABLE II  
VARIATION OF TOXICITY WITH RATE OF ADMINISTRATION OF COCAIN—AVERAGE

CATS	STRENGTH OF SOLUTION PER CENT COCAIN	RATE OF INJECTION GM. PER MIN.	LETHAL DOSE COCAIN—GM. PER KILO.	
			RESPIRATION STOP	HEART STOP
3	$\frac{1}{2}$	.0115	.0128	.0213
3	$\frac{1}{4}$	.0057	.0151	.0194
3	$\frac{1}{8}$	.0028	.0208	.0231
3	1	.0230	.0150	.0359

cocain with adrenalin could hardly help being depressing. Therefore work was directed to determine whether adrenalin is an antidote for cocain poisoning or a substance increasing its toxicity.

#### EXPERIMENTAL WORK

Cats were used as subjects. They were given enough ether to quiet them and were then injected intraperitoneally with  $\frac{3}{4}$  c.c. of a 25 per cent solution of chloretone in olive oil, for each kilogram of body weight. The femoral vein was exposed and a canula inserted into it. Connected with the canula there was a motor driven machine which injected the solution at the uniform rate of 2.3 c.c. per minute.

The first point to be determined was the effect of the rate of injection on the lethal dose. The first three cats were given a 1 per cent solution of cocain in normal salt solution. The lethal dose was measured by the time required to stop respiration and heart beat. The second three cats were given a half per cent solution of cocain. The third group was given a quar-



ter per cent solution and the fourth group an eighth per cent solution. The results are given in Table I. The average results are given in Table II.

The second part of the problem was to determine whether adrenalin increased or decreased the lethal dose of cocain. Seven groups of three cats each were used for this purpose. The first group of cats were given normal salt solution containing one-fourth per cent cocain and 0.2 per cent adrenalin

TABLE III  
EFFECT OF ADRENALIN ON TOXICITY OF COCAIN

CAT	PER CENT ADREN.—WITH COCAIN		LETHAL DOSE COCAIN—GM. PER KILO.		
			RESPIRATION	STOP	HEART STOP
1	None	1/4%	.0144		.0212
2	None	"	.0168		.0190
3	None	"	.0141		.0181
4	0.2	"	.0144		.0194
5	0.2	"	.0175		.0213
6	0.2	"	.0139		.0190
7	0.4	"	.0126		.0149
8	0.4	"	.0173		.0219
9	0.4	"	.0125		.0227
10	1.0	"	.0117		.0165
11	1.0	"	.0089		.0115
12	1.0	"	.0104		.0137
13	5.0	"	.0090		.0116
14	5.0	"	.0080		.0109
15	5.0	"	.0060		.0110
16	10.0	"	.0065		.0106
17	10.0	"	.0051		.0096
18	10.0	"	.0073		.0172
19	None	1/8%	.0195		.0214
20	None	"	.0225		.0263
21	None	"	.0196		.0215
35	1.0	"	.0093		.0093
36	1.0	"	.0144		.0154
38	1.0	"	.0111		.0115

TABLE IV  
EFFECT OF ADRENALIN ON TOXICITY OF COCAIN—AVERAGE

PER CENT ADRENALIN WITH COCAIN		LETHAL DOSE OF COCAIN—GRAMS PER KILO.	
		RESPIRATION STOP	HEART STOP
0.00	1/4	0.0151	0.0194
0.20	1/4	0.0153	0.0199
0.40	1/4	0.0141	0.0198
1.00	1/4	0.0103	0.0139
5.00	1/4	0.0077	0.0107
10.00	1/4	0.0063	0.0129
0.00	1/8	0.0208	0.0231
1.00	1/8	0.0116	0.0121

(1:1000). The second group received the same except that the solution contained 0.4 per cent adrenalin. The third group got 1.0 per cent adrenalin. The fourth group was given 5.0 per cent adrenalin with cocain and the fifth group 10 per cent adrenalin with cocain. The sixth group received 15 per cent adrenalin and no cocain. The seventh group was given 1.0 per cent adrenalin and one-eighth per cent cocain. These results are give in Table III. The average results are given in Table IV.

## DISCUSSION

The measurement of the minimal lethal dose was made by determining the moment of the stoppage of respiration and also of the heart. It was, at first, thought that the failure of the heart would be the best indication that injection of the lethal dose had been completed. However, it was found that the actual time of cessation of beating could not be determined without exposure of the heart and that the heart-beat could not be detected by touch or vision equally well in all individuals. Consequently, the time when regular respiration stopped was considered as the best indicator that the lethal dose had been administered. The dose calculated from the time of the arrest of the heart-beat is included here chiefly as a check on the other.

In Table I, it may be noted that there was a rather wide variation between individuals of the group. This variation is to be expected as it is a well-known fact that among people there is a wide range of susceptibility to cocain.

Table II includes the average of the results of the group of animals given the cocain at different rates. As the concentration of the solution of cocain decreased from 1 to  $\frac{1}{8}$  per cent, or as the rate of injection of cocain decreased from .0230 to .0028 grams per minute, the lethal dose first dropped and then gradually raised to its highest point at  $\frac{1}{8}$  per cent concentration. The lethal dose for the 1 per cent solution was higher than that for the  $\frac{1}{2}$  per cent solution, because the rate of introduction was greater than the rate at which the toxic action of cocain developed in these animals. In other words, when the respiration stopped in the first group of cats there must have been a certain amount of cocain in those cats that had not had time to take any part in killing them. The increasing lethal dose with a decreasing rate of introduction of cocain, from the  $\frac{1}{2}$  per cent to  $\frac{1}{8}$  per cent, was probably due to the elimination of a part of the cocain by the time respiration stopped. According to measurements made with reference to respiration,  $\frac{1}{2}$  per cent cocain solution injected at the rate of 2.3 c.c. per minute seemed to be the most effective. There seemed to be with this rate of introduction the least amount of eliminated cocain and the least amount of cocain unutilized. Therefore, for cats weighing between two and three kilos, the minimal lethal dose under the described conditions is .0125 grams of cocain hydrochloride per kilogram of body weight. This agrees very closely with the M.F.D. for rabbits as stated by Sollman<sup>8</sup> which is given as .01 to .022 grams per kilo.

The effect of adrenalin on the lethal dose of cocain is shown for individuals in Table III. As for cocain alone, there was a wide individual variation for the combination of adrenalin and cocain. The average group results are given in Table IV, from which a comparison of the drug effects can best be made. Inasmuch as the two cats, injected with 15 per cent adrenalin for 35 to 40 minutes at the rate of 2.3 c.c. per minute, were apparently uninjured, it was considered that the toxic effect of adrenalin alone, when in concentrations of 10 per cent or less and injected for periods of less than 15 minutes, was negligible. The addition to  $\frac{1}{4}$  per cent cocain solution of increasing amounts of adrenalin from .4 to 10 per cent gradually increased the toxicity

of the solution. The addition of .2 per cent adrenalin seemed to have little or no effect on the toxicity of the cocain solution. The addition of .4 per cent adrenalin increased the toxicity only about 7 per cent. However, the addition of 1, 5, and 10 per cent of adrenalin increased the toxicity of the mixtures approximately 47, 95, and 140 per cent.

The effect of adding 1 per cent adrenalin to a  $\frac{1}{8}$  per cent cocain solution was determined. Without adrenalin the lethal dose of cocain was .0208 grams per kilo and with the adrenalin it was .0116. This increase in toxicity of the solution was approximately 79 per cent. It is an interesting fact that the minimal lethal dose of cocain in a 1 per cent adrenalin is approximately the same whether the cocain is  $\frac{1}{8}$  or  $\frac{1}{4}$  per cent. This means simply that, other things being equal, the increased amount of adrenalin injected with the  $\frac{1}{8}$  per cent cocain solution made up for the loss in toxicity due to slower rate of introduction of the cocain in the  $\frac{1}{8}$  per cent solution.

#### SUMMARY AND CONCLUSIONS

Cats were given a light dose of chloretone. Cocain hydrochloride in concentrations varying from  $\frac{1}{8}$  to 1 per cent in normal salt solution was injected mechanically into the leg veins. The rates of introduction of the cocain varied from .0028 to .0230 grams per minute, per kilogram of body weight. As the rate of introduction of cocain increased the minimal lethal dose of cocain first decreased and then gradually increased up to the end of the series. The minimal lethal dose of cocain hydrochloride for chloretoned cats under these conditions is .0128 grams of cocain per kilo of body weight.

A series of groups of cats were injected with a  $\frac{1}{4}$  per cent solution of cocain hydrochloride to which was added increasing amounts of adrenalin ranging from .2 to 10 per cent. With the increase in the concentration of adrenalin there was a gradual increase from 7 to 140 per cent in the toxicity of the mixture. In addition to this series a group of animals was given a normal saline solution containing 1 per cent adrenalin and  $\frac{1}{8}$  per cent cocain hydrochloride. It was found that the adrenalin increased the toxicity of the solution 79 per cent. Adrenalin in a concentration of 15 per cent injected at a rate of 2.3 c.c. per minute could not be made to kill cats.

Briefly stated the conclusions are as follows:

1. The M.F.D. of cocain for cats varies with the rate of administration.
2. The absolute M.F.D. of cocain hydrochloride for chloretoned cats is .0128 grams per kilo of body weight.
3. Adrenalin markedly increases the toxicity of cocain.

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# A METHOD FOR THE ISOLATION OF WHITE BLOOD CELLS\*

BY PAUL SZILARD, M.D.

## INTRODUCTION

EXPERIMENTAL work with white blood corpuscles is rendered exceedingly difficult and in many instances impossible by the circumstance that we do not possess a reliable method to separate the cells in bulk from other tissue. The generally applied method, i.e., the injection of a sterile solution of aleuronat into the abdominal cavity of a guinea pig, produces a pathologic condition which may well affect the white cells, while furthermore, the exudate which gathers in the abdominal cavity consists almost entirely of polynuclear neutrophile granulated leucocytes, and very few monocytes and lymphocytes are to be found. White cells may also be obtained by differential centrifugation, but always some red cells and thrombocytes are mixed therewith. The need for a reliable method to obtain leucocytes is shown by the utilization that has been made of various fluids in which they are abundant, i.e., the pus of fresh abscess (Neufeld and Rimpau), gonorrheal secretion from the urethra (Loewenstein), cystitic urine (Loewenstein), meningitic exudate (Davis), tuberculous sputum (Loewenstein).

The writer has worked out a simple means whereby white blood cells can be isolated almost quantitatively from a small volume of blood.

The essential point of the procedure is the destruction of the red blood corpuscles and the thrombocytes in a manner which will leave the white cells unimpaired. Acetic acid, usually applied for dissolving red blood corpuscles, is useless here, for two reasons: First, it injures the white blood cells inasmuch as it coagulates the proteids of their nuclei; and in addition, after hemolysis and centrifugation, the sediment contains not only the white cells, but also the remains of the erythrocytes. Hemolysis with distilled water is useless for the same reason.

Experiments were undertaken with acids in different concentrations, i.e., HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, and with organic acids such as citric, tartaric, formic acid, etc.

While testing various combinations it was observed that in a certain mixture of acetic and tartaric acid the white blood cells had scarcely suffered at all; their nucleoproteids had undergone relatively little coagulation and the number of destroyed cells was small. It seemed probable that the tartaric acid served partly to reduce the coagulating effect of the acetic acid.

An attempt was now made to neutralize the free acid remaining after hemolysis had been accomplished by the combined acetic and tartaric acids.

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The result was encouraging, for although some red blood corpuscles were present in the sediment, their number was small. When the mixture was rendered not merely neutral but slightly alkaline, only white blood cells were found in the deposit after centrifugation.

#### PROCEDURE

The following are the solutions required:

1. 2.5 per cent solution of acetic acid.
2. 2 " " " " tartaric acid.
3. 2 " " " " potassium hydroxide.
4. Loeki's solution.

Blood taken from the cubital vein by puncture or by syringe is defibrinated by gentle shaking with glass beads. Twenty-five to 30 c.c. will yield a goodly quantity of white blood cells. The blood should if possible be used

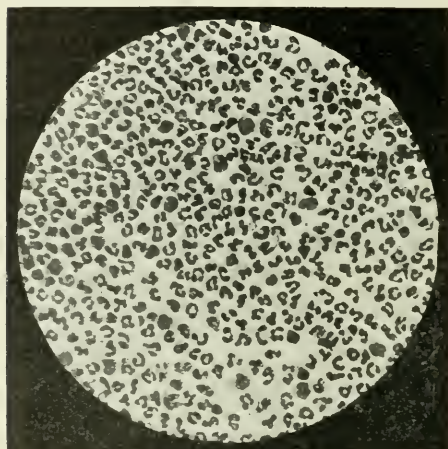


Fig. 1.—The white blood cells of a healthy man isolated by the above described method. Zeiss apparatus. Apochrom. object.: 8 mm. Project. ocul.: 2 mm. Tubus length: 160 mm.

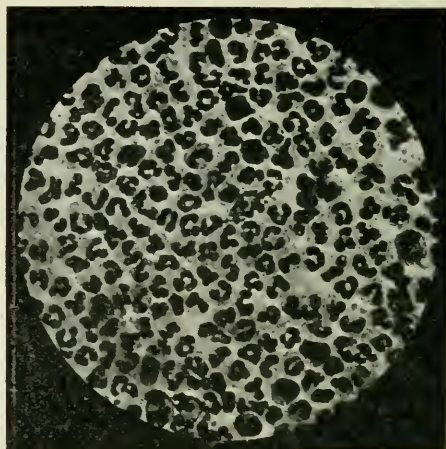


Fig. 2.—The same as Fig. 1. Apochrom. object.: 4 mm. Project. ocul.: 2 mm. Tubus length: 160 mm.

immediately after defibrination, and under no circumstances should it be allowed to stand longer than from 2 to 3 hours before using.

A mixture of acetic and tartaric acid in the proportion of 4:1 is employed for hemolysis. Of this mixture only the quantity required for complete hemolysis should be employed. This minimal amount of acid can be determined by preliminary experiments. In my experience, 12 c.c. of acetic and 3 c.c. of tartaric acid are required for 10 c.c. of blood.

The quantity of alkali necessary to neutralize the acid mixture to phenolphthalein should also be determined, and for each 10 c.c. of blood 1 c.c. more than the quantity of alkali required for neutralization will be needed. If any of the stock solution deteriorates through bacterial infection—and this is frequently the case with the tartaric acid—it must be titrated again.

The acid mixture is poured quickly into the blood with which it is

mixed by gentle rotation until complete hemolysis has taken place, as indicated by the varnish-like appearance of the laked blood. A too prolonged treatment with acid is undesirable, since it causes the white cells to deteriorate. The alkali is now poured rapidly into the tube and well mixed, and the whole transferred to a centrifuge tube. If the mixture is brown in color or shows turbidity when held against the light, it should be discarded. This happens when an insufficient amount of alkali has been employed.

The centrifugation should not be continued for longer than five minutes, as it is injurious to the white cells. But more important than the period of centrifugation is the rate of speed, which should not exceed 1,000 to 1,500 revolutions per minute, since very rapid centrifugation damages the cells.

After centrifugation the cells will be seen at the bottom of the tube as a white agglutinated mass, while the supernatant fluid is homogeneous, transparent, and bright purplish-red in color. It should be poured off and the sediment suspended in Locke's solution and washed with it once or twice at a low speed of the centrifuge. Ordinary sodium chloride solution should not be employed for washing, as it is injurious to the leucocytes.

When a portion of the washed cell sediment is spread on a slide and stained with May Grünwald and Giemsa stains by Pappenheim's method, only an occasional red cell is to be seen. At first sight there would appear to be present a greater number of young leucocytes and monocytes than we find in the microscopic film made from blood taken from the tip of the finger. But that is an erroneous impression, due to the great number of cells massed together. A differential count shows the usual proportion to exist amongst the leucocytes. Typical pictures are shown in Figs. 1 and 2.

When the white corpuscles are suspended in a little serum, or better, in their own plasma, and incubated for from  $\frac{1}{2}$  to one hour in a hanging drop, their ameboid movement is clearly visible—evidence that the cells are alive. Directly after centrifugation, no movement is seen or is seen only rarely.

In experiments with living leucocytes great attention should be paid to the apparatus. Glassware, centrifuge tubes, solutions, etc., should be kept throughout in the thermostat at 37° C. For microscopic examination a warm stage is recommended, for the leucocytes are rather sensitive to external influences. The blood if not used directly should also be kept in the thermostat, under no circumstances in the ice box. Great care should be paid to sterility.

Even under the best of circumstances, some 20 per cent to 30 per cent of the white cells are spoiled, on account of the coagulation of their nucleoproteids. The remaining white blood corpuscles can be kept alive for 24 hours or even longer; they are even capable of phagocytosis.

The method is equally suitable for the isolation of human and of animal white blood cells; but the quantity of acids and alkali required differs with the species. To hemolyze 10 c.c. defibrinated sheep's blood requires 8 c.c. of a 2.5 per cent solution of acetic acid plus 2 c.c. of a 2 per cent solution of

tartaric acid; for 10 c.c. of defibrinated horse's blood, 10 c.c. of acetic acid plus 2.5 c.c. of tartaric acid is needed; and for 10 c.c. defibrinated ox blood, 10 c.c. of acetic acid plus 2.5 c.c. of tartaric acid; while for 10 c.c. defibrinated swine's blood, 12 c.c. of acetic acid plus 3 c.c. of tartaric acid must be used. The quantity of a 2 per cent solution of potassium hydroxide must be varied to suit the case.

The technic of this simple method can be thoroughly mastered in the course of a few days. By it the plasmodium of malaria is readily obtained.

## CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

BY A. LEVINSON, M.D., CHICAGO, ILL.

(Continued from page 588.)

### CEREBROSPINAL FLUID

#### INDICATIONS FOR REMOVAL OF FLUID.—

- |               |   |   |
|---------------|---|---|
| For Diagnosis | { | Suspicious meningitis<br>Suspicious poliomyelitis<br>Hemorrhage of the brain<br>Neurosyphilis<br>Coma<br>Convulsions  |
| For Treatment | { | (a) Relief of intracranial pressure<br>Meningitis<br>Meningism<br>Poliomyelitis<br>Encephalitis<br>Hemorrhage of the brain<br>Delirium<br>Convulsions<br><br>(b) Intraspinal injections<br>1. Serum Treatment<br>Antimeningococcus<br>Pneumococcus Type I<br>Influenza<br>Poliomyelitis<br>Tetanus antitoxin<br>Horse serum<br><br>2. Chemicals<br>Neosalvarsan<br>Mercury<br>Optochin<br>Magnesium sulphate<br>Novocain<br>Adrenalin<br><br>(c) Miscellaneous<br>General edema<br>Diabetes insipidus |



## METHODS OF REMOVING FLUID

One of three routes may be used to withdraw cerebrospinal fluid from a human being: (1) Spinal, (2) Ventricular, (3) Cistern or occipito-atloid. Of these three routes, the spinal is the simplest and most advisable. Next to it is the ventricular route, and next to it, and the most dangerous route, is the cistern or occipito-atloid. Whenever possible, therefore, a spinal puncture should be done for the withdrawal of the cerebrospinal fluid from the body. Because of its importance, spinal puncture will be discussed first.

**TECHNIC OF SPINAL PUNCTURE.**—The needle for spinal puncture in children should be of a wide lumen, otherwise, the thick fluid often encountered in suppurative meningitis may occlude the opening of the needle. There are several makes of spinal puncture needles on the market, most of them being  $2\frac{1}{2}$  to 3 inches in length and 0.2 to 0.6 mm. in thickness. Each needle is fitted with a stylet. There are also needles with stopcocks made to fit various manometers used for measuring the pressure of the fluid.

The test tubes for collecting the fluid should be small, sterile and chemically clean, otherwise the interpretation of the results obtained by examination may be misleading.

The preparation for spinal puncture should be the same as for any other operation. The needle should be boiled, the patient's skin should be washed with alcohol and iodine and the physician should scrub his hands and wear gloves during the puncture.

The puncture should be done with the patient on a table or cart, a bed not being steady enough. All punctures should be performed with the patient lying down, preferably on his right side. The patient's body should be bent, so as to separate the intravertebral spaces. The needle should be introduced directly into the median line in very young children, and a few millimeters away from the median line in older children. The space of choice for the introduction of the needle is between the second and third interspace, although in children one may go one space higher without fear. A horizontal line drawn at the level of the crest of the ilium will usually strike the desired interspace.

After puncturing the skin, the needle should be directed forward and slightly upwards, until a snap is felt, which usually indicates the puncturing of the dura. The stylet should then be removed and the fluid, if obtainable, emptied into the test tubes. In case a manometer is used, the stopcock should be turned to prevent the escape of fluid, the manometer attached, and the pressure measured before the fluid is allowed to run into the test tubes.

It has been found best to use three or four test tubes for the collection of the fluid, so that if the patient moves around during the latter stage of the puncture and the fluid becomes bloody, as often happens, the first or second tube could still be used for examination. After the desired amount of fluid has been obtained, the stylet should be reintroduced into the needle, and the needle pulled out from the patient's body. The wound should be closed with collodion.

Rest in bed should be insisted upon after every spinal puncture. While



it does not always prevent headache, if it does occur, the headache is not so severe as when the patient is up and around.

**FAILURES IN SPINAL PUNCTURE.**—The two most important and most frequent failures are (1) inability to obtain fluid and (2) obtaining bloody fluid. While there are cases where there is very little or no fluid in the spinal canal, such as in marked atrophy of the spinal cord and in obstruction of the canal by a tumor, the average dry tap is due to the fact that the operator has not reached or has passed beyond the spinal canal. In very fleshy children it is hard to feel the intervertebral spaces. Failure to obtain fluid is therefore more frequent in fat than in slender children.



Fig. 23.—Photograph showing ventricular puncture in infant by way of the anterior fontanelle. Dark circular spot shows needle in position. (Front view.)

In deformities of the spine, due to rickets or tuberculosis, it may be impossible to reach the spinal canal. In such cases, it may be necessary either to introduce the needle obliquely to reach the spinal canal, or, to resort to a ventricular puncture.

Obtaining bloody fluid is possibly the most frequent mishap in spinal puncture. It happens to both the inexperienced and the experienced. The blood is due to injury produced to the plexus of veins in the canal. Once blood is obtained the fluid cannot be used for cell count or any chemical tests. It may, however, still be used for bacteriologic purposes. A Wassermann

test may be performed on bloody fluid, although at times the blood makes the fluid anticomplementary. If clear fluid is desired for examination, no new puncture should be done for several days after blood has been obtained, as it takes three days for blood to be absorbed from the spinal canal. However, when it is necessary to give serum intraspinally, blood obtained on spinal puncture does not counterindicate the procedure.

Death after spinal puncture has been reported in the literature. Death in those cases was seemingly due to the withdrawal of too great a quantity of fluid, thereby allowing parts of the brain to descend to the foramen magnum and become obstructed there. With the exception of a tumor of the

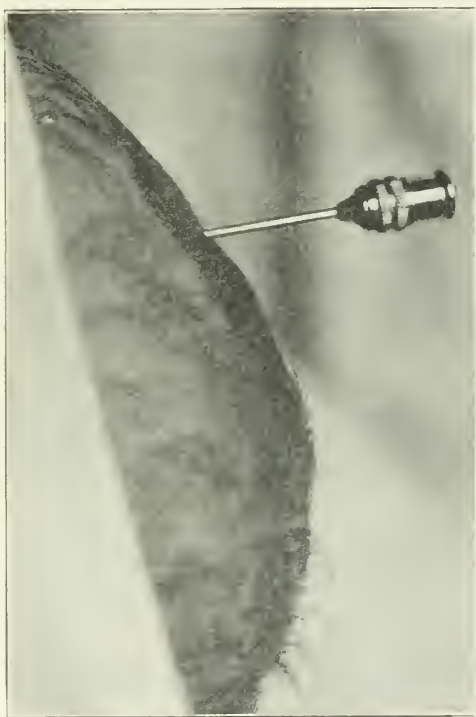


Fig. 24.—Photograph showing ventricular puncture in infant. (Side view.)

cerebellum, no condition counterindicates spinal puncture, the possibility of death by puncture being minimal. I have done many punctures in cases of brain tumor without a single complicating death.

Shock is not an infrequent complication. A hypodermic injection of atropine usually relieves it.

Injury to the aorta by spinal puncture has been reported, but is very uncommon. Breaking off the needle is not an infrequent complication in adults, but seldom occurs in children.

Headache after a spinal puncture is a common occurrence, but usually disappears in a day or two, especially if the patient stays in bed.

Radiating pain in the lower extremities takes place if filaments of nerves

have been touched in the spinal canal by the needle. The pain, however, does not last long.

Edema of the skin in the lumbar region has been observed after repeated punctures. The edema, however, subsides in a few days.

**VENTRICULAR PUNCTURE.**—*Indications.*—Ventricular puncture in infants is not associated with any danger, yet it should not be performed unless lumbar puncture has failed repeatedly. Where the latter has failed either for the purpose of the removal of cerebrospinal fluid or for the introduction of serum, ventricular puncture should be performed without hesitation.

*Technic.*—The hair around the anterior fontanelle should be shaved and alcohol and iodine applied. The operation proper varies with the age of the child. In infants the operation is very simple. In older children, in whom the anterior fontanelle is closed, the procedure is complicated and requires many surgical instruments.

The technic in infants is as follows: The patient is placed in the recumbent posture on a table, with the head at the end of the table. The head, shaved and washed with alcohol, is steadied by an assistant. A regular spinal puncture needle is now introduced a few millimeters to one side of the midline of the anterior fontanelle. The needle is directed forward and slightly downward to a depth of 1 to 1½ inches. In hydrocephalic children ½ inch suffices. The stylet is now removed and fluid is collected into one or more test tubes (Figs. 23 and 24). If no fluid is obtained, the needle should be withdrawn and reintroduced, but should not be manipulated unduly while in the brain, in order to avoid damage to the brain tissue. If no fluid is obtained by the fontanelle route, and if the bones of the skull are still separated, the needle may be introduced between the frontal and parietal bone 1 to 1½ inches deep.

#### CISTERN PUNCTURE

If both the spinal and ventricular routes have failed for the purpose of obtaining cerebrospinal fluid, or for the introduction of serum, cistern puncture may be employed. The technic, originally described by Wegeforth, Ayers and Easick is as follows:

A spinal puncture needle is introduced into the midline of the back of the neck just above the spine of the axis. The glabella and the upper edge of the external auditory meatus may be used as a landmark for the insertion of the needle, for a plane passed through them to the back of the neck will pass also through the occipito-atloid ligament. In thin individuals a deep depression can be palpated between the occipital protuberance and the spine of the axis. This depression serves as another landmark. The needle is introduced at a depth of 3 to 5 cm., the average being 4 cm.

The preparation for the puncture and the collection of the fluid are the same as in spinal or ventricular punctures.

#### METHODS OF EXAMINATION

The fluid should be examined for physical, chemical, physiochemical, bacteriologic and immunologic alteration.



Fig. 25.—Lange gold chloride reaction in a case of tuberculous meningitis.







Fig. 26.—Lange gold chloride reaction in a case of meningococcus meningitis.



**AMOUNT.**—Normally, only five to ten c.c. of cerebrospinal fluid can be removed by spinal puncture. In nearly all pathologic cases, the amount of cerebrospinal fluid is increased. In acute infections, whether due to inflammation or only to irritation of the meninges, the amount is greatly increased and one can often remove as high as 30 to 40 c.c. in one sitting. In chronic inflammation of the meninges or of the brain, the amount is also increased. Only in tumor of the cord and in some cases of obstructive hydrocephalus is the amount decreased. Seldom does one see a case where no fluid at all can be removed from the spinal canal and which is not due to faulty technique. Such may be the case in tumor of the cord or in spina bifida.

**PRESSURE.**—Normally, the pressure of the cerebrospinal fluid, when measured as it escapes from the canal, is 40 to 90 mm. of water high in the recumbent posture in children. In acute infections or irritations of the meninges the pressure may go up as high as 300 or 400 mm. of water. In cases of obstruction of the cord, the pressure may be lessened.

**COLOR.**—Normal cerebrospinal fluid is colorless. Many pathologic conditions do not change the color of the fluid, such for instance is the case in encephalitis, poliomyelitis and tuberculous meningitis. In some conditions, however, the fluid changes color. In all suppurative meningitis for instance, the fluid is turbid. In jaundice, the fluid is greenish-yellow; in tumors of the cord, the fluid is deep yellow, and is known as xanthochromia. In hemorrhage of the brain, the fluid is bright or dark red, in the early stages, but may be colorless after a few weeks.

**SEDIMENT OR PELLICLE.**—Normal fluid forms no sediment when allowed to stand. In meningitis, however, a pellicle or sediment often forms in the fluid on standing. In suppurative forms of meningitis, the pellicle forms within a few minutes. In tuberculous meningitis, it usually takes 12 to 24 hours for the sediment to form.

**GLOBULIN.**—Normal fluid contains only a small amount of protein. All acute and chronic inflammation of the meninges increases the protein in the fluid.

The following are the most simple tests for the determination of the increase of globulin.

**Pandy.**—One drop of cerebrospinal fluid is added to one or two c.c. of a concentrated solution of carbolic acid (1 part phenol crystals to 15 parts of water). A bluish white cloud forms in the test tube if globulin is present in excess of normal. When mixed, the solution becomes turbid. If the globulin is not increased the solution remains clear.

**Ross-Jones.**—Two-tenths to 0.5 c.c. of a saturated ammonium sulphate solution is poured into a small test tube. An equal amount of cerebrospinal fluid is floated upon it by running it down the side of a slanted tube. A white ring develops in a few seconds to 2 or 3 minutes at the point of contact, if the globulin in the fluid is increased.

**Noguchi.**—Two-tenths c.c. of cerebrospinal fluid is poured into a small test tube, and 0.5 c.c. of a butyric acid solution (5 c.c. of butyric acid to 45 c.c. physiologic salt solution) is added to the fluid. The mixture is boiled for a



few seconds and 0.1 c.c. of NaOH (normal 4 per cent aqueous solution) is added to it and it is again boiled for a few seconds. If the globulin in the fluid is increased, a fine or coarsely granular, flocculent deposit forms in from one to ten minutes. If no coarse flocculi appear within two hours, even if a slight opalescence is present the globulin is not increased.

**SUGAR.**—Normally, cerebrospinal fluid contains 0.08 to 0.1 per cent of sugar. In suppurative meningitis, especially in the meningococcus form, the sugar is either decreased or absent from the fluid. An absence of sugar in the fluid, therefore indicates meningitis. Although not very accurate, Fehling's reduction test will answer the purpose.

**THE LANGE GOLD CHLORIDE TEST.**—The gold chloride test of Lange may be considered of great value in the diagnosis of pathological conditions of the cerebrospinal fluid. Normally, the fluid does not change the ruby red color of the original gold chloride solution. In luetic infections of the central nervous system, one or more of the first five tubes in the series, is changed in various degrees, from violet to colorless. In tuberculous meningitis the fifth, sixth and seventh tubes are usually affected. In suppurative meningitis one or more of the last five tubes change colors. (Figs. 25 and 26.)

The technic of making up the gold chloride solution is rather difficult, some solutions producing color changes even with normal fluids, and others producing no changes even with distinctly luetic fluids. Care must be taken to see that the solution is transparent, of neutral reaction, and that it is precipitated by 1.7 c.c. of 1 per cent NaCl solution in one hour. There are several modifications of the original Lange technic of preparing colloidal gold solution, but we believe the following technic to be very satisfactory.

The method is as follows: To 1000 c.c. of doubly distilled water, 10 c.c. of a 1 per cent gold chloride solution and 7 c.c. of a 2 per cent solution of potassium carbonate are added. The mixture is heated to 90° C., stirred vigorously and 5 c.c. of a 1 per cent formaldehyde solution is added. The solution should at once assume a red color.

The method of testing cerebrospinal fluid with the above solution is as follows: 0.2 c.c. of cerebrospinal fluid is introduced into the first one of a series of 10 test tubes containing 1 c.c. of 0.4 per cent sodium chloride. Ten dilutions of the cerebrospinal fluid are now made, by taking 1 c.c. of the first tube and transferring to the second, then 1 c.c. of the second and transferring to the third, etc., the dilutions thus ranging from 1 to 10 to 1 to 5120. An eleventh tube containing 1 c.c. of 0.4 per cent sodium chloride without cerebrospinal fluid may be used as a control. It is also advisable to run another gold chloride test on a fluid known to be normal. To each tube 5 c.c. of the colloidal gold reagent (1 per cent solution of gold chloride, 2 per cent solution of  $K_2CO_3$ , 1 per cent solution of formalin) is added.

**CELLULAR ELEMENTS.**—Normal fluid contains one to six cells per cubic mm. all of which are small lymphocytes. In infections of the meninges and occasionally in irritation of the meninges, the number of cells are increased. The more acute the infection, the greater the number of cells.

The type of cell differs with the pathologic condition. In tuberculous

TABLE XII  
CEREBROSPINAL FLUID FINDINGS IN VARIOUS CONDITIONS

CONDITION	ORGANISM	COLOR	AMOUNT (Number of c.c. easily re- moved)	PRESSURE	SEDIMENT	CELLS (Number per c. mm. and type)	PERMAN- GANATE INDEX	GLOBULIN INCREASE	SUGAR	WASSER- MANN	LANGE	SPECIAL TESTS
Normal	None	Clear	5-10	90 mm. of water in child, 150 mm. in adult	None	1-6 Small lympho- cytes	Less than 2	No	0.05- 0.1%	Negative	Negative	
Uremia	None	Clear	Normal or Increased	Increased	None	Normal	2 or over	slight or marked	Normal	Negative	Negative	Urea may be increased. Chlorides occasion- ally increased.
Diabetes	None	Clear	Normal	Normal	None	Normal	2 or over	No	Above 0.1%	Negative	Negative	Acetone may be present.
Skull frac- ture (without meningeal hemorrhage)	None	Clear	Normal or greatly increased	Normal or Increased	None	Normal	Less than 2	No	Normal	Negative	Negative	
Meningeal hemorrhage (a) bloody	None	Bloody	Increased	Increased	Bloody	Many red and white	Above 2	Yes	Normal	Negative	Negative	Cholesterol present. Serum albumin in large quantities.
(b) clear (Old standing)	None	Clear	Normal or Increased	Increased	None	5-10 small lympho- cytes	2 or over	Slight	Normal	Negative	Negative	Cholesterol present. Serum albumin may be present in large quantities.
Lateral sinus Thrombosis	None	Clear	Normal or Increased	Increased	None	Normal	Normal	No	Normal	Negative	Negative	
Brain tumor	None	Clear	10-15	Slightly increased	None	10-15 Small or large lympho- cytes	Normal	Slight	Normal	Negative	Occa- sionally 044230- 0000	Inhibition of hemol- ysis described by Hauptmann. Increased cholesterol.

TABLE XII—CONTINUED.  
CEREBROSPINAL FLUID FINDINGS IN VARIOUS CONDITIONS

CONDITION	ORGANISM	COLOR	AMOUNT	PRESSURE	SEDIMENT	CELLS	PERMAN- GANATE INDEX	GLOBULIN INCREASE	SUGAR	WASSER- MANN	LANCZ	SPECIAL TESTS
Brain abscess (a) not ruptured	None	Clear	Slightly increased	Slightly increased	None	10-15 Small lympho- cytes Occa- sionally large lympho- cytes	Normal	Slight	Normal	Negative	Occa- sionally 044430- 0000	
(b) ruptured	Any or- ganism	Turbid	Increased	Increased	Yes	Several thousand poly- morpho- nuclear leuco- cytes	Above 2	Marked	Normal	Negative	000000- 444	
Spinal cord tumor	None	Yellow (Xantho- chromia)	De- creased	De- creased	Coagulates sponta- neously	None or 1-2	None or Above 2	Marked	Normal	Negative		
Syphilis of central nerv- ous system (a) Gen. Paresis	None	Clear	10-40	Increased	None; Occasion- ally small floculi	40-200 Small lympho- cytes	Normal	Marked	Normal	Positive	555550- 0000 (Paretic curve)	
(b) Cerebro- spinal syph- ilis	None	Clear	Increased 15-30	Increased	None	100-20 Small lympho- cytes	Normal	Yes	Normal	Positive	005555- 0000	
(c) Tabes dorsalis	None	Clear	Increased	Increased	None	60-80 Small lympho- cytes	Normal	Yes	Normal	Positive	005550- 0000 (Tabetic curve)	

TABLE XII—CONTINUED.  
CEREBROSPINAL FLUID FINDINGS IN VARIOUS CONDITIONS

CONDITION	ORGANISM	COLOR	AMOUNT	PRESSURE	SEDIMENT	CELLS	PERMAN- GANATE INDEX	GLOBULIN INCREASE	SUGAR	WASSER- MANN	LANGE	SPECIAL TESTS
(d) Juvenile paresis	None	Clear	Increased	Increased	None	40-80 Small lympho- cytes	Normal	Yes	Normal	Positive	55550- 0000	
Meningism	None	Clear	Increased	Increased	None	Normal or slight ly in- creased	Normal	No or slight	Normal	Negative	Negative	
Epidemic encephalitis	Minute filterable virus de- scribed by some authors	Clear	10-30	Increased	None	10-20 Small mono- nuclear	Normal	Slight	Normal	Negative	Negative	
Anterior poliomyeli- tis	Micrococ- cus de- scribed by some au- thors	Clear	Increased	Increased	None	10-100 poly- morpho- nuclear very early, mono- nuclear after sec- ond day	Normal	Yes	Normal	Negative	Lactic zone ear- ly. Meningi- tic zone later	
Tuberculous meningitis	Tubercle bacilli	Clear or slightly opales- cent	Increased	Increased	Fine pellicle	30-400 Mono- nuclear	Above 2	Yes	Normal or less	Negative	Between lactic and menin- gitic zones. (000023- 3000)	(1) Cataphoresis to the anode. (2) Sulphosalicylic precipitate 3-6 mm. Biehl's mercury 6-20 mm. (3) Guinea pig inoc- ulation shows tubercles



TABLE XII—CONTINUED.  
CEREBROSPINAL FLUID FINDINGS IN VARIOUS CONDITIONS

CONDITION	ORGANISM	COLOR	AMOUNT	PRESSURE	SEDIMENT	CELLS	PERMAN- GATE INDEX	GLOBULIN INCREASE	SUGAR	WASSER- MANN	LANGÉ	SPECIAL TESTS
Meningococ- cus menin- gitis	Meningo- coccus	Turbid	Increased	Markedly Increased	Thick, Varying with stage of disease	50-8000; 95% Poly's; Endo- thelial cells present	Above 3	Marked	Absent or greatly dimin- ished	Negative	Menin- gitic zone 00000- 45555	(1) Cataphoresis to the cathode. (2) Agglutination of bacteria by specific serum. (3) Precipitation of fluid with anti- men. serum (4) Sulphosalicylic acid 7-20 mm., Bichloride 5-6 mm.
Pneumococ- cus menin- gitis	Pneumo- coccus	Turbid	Increased	Increased	Present	50-8000 98% Poly's	Above 2	Yes	Normal or dimin- ished	Negative	Menin- gitic zone	Agglutination. Sulphosalicylic 7-20 mm.; Bichloride 5-6 mm.
Strepto- coccus menin- gitis	Strepto- coccus	Turbid	Increased	Increased	Yes	50-1000 98% Poly's	Above 2	Yes	Normal or dimin- ished	Negative	Menin- gitic zone	Sulphosalicylic 7-20 mm. Bichloride 5-6 mm.
Influenza meningitis	Influenza bacillus	Turbid	Increased	Increased	Yes	50-1000 98% Poly's Occa- sionally 50% lympho- cytes	Above 2	Yes	Normal or dimin- ished	Negative	Menin- gitic zone	Sulphosalicylic 7-20 mm. Bichloride 5-6 mm. Indol test described.
Other Meningitis	Specific organism	Turbid	Increased	Increased	Heavy	50-1000 98% Poly's	Above 2	In- creased	Normal or dimin- ished	Negative	Menin- gitic zone	

meningitis, in certain stages of poliomyelitis, the cells are mainly lymphocytes. On the other hand, the cells in all suppurative meningitis are mainly polymorphonuclear in type. In addition to the polymorphonuclear cells, however, there are also endothelial cells. This type of cell is particularly predominant in meningococcus meningitis. The same is true with the phagocytes which are present in large number in meningococcus meningitis. In tumor of the brain large lymphocytes may be present in considerable number. (Fig. 27.)

*Method of Counting Cells.*—A special counting apparatus has been devised by Fuchs and Rosenthal for the counting of cells in cerebrospinal fluid. This

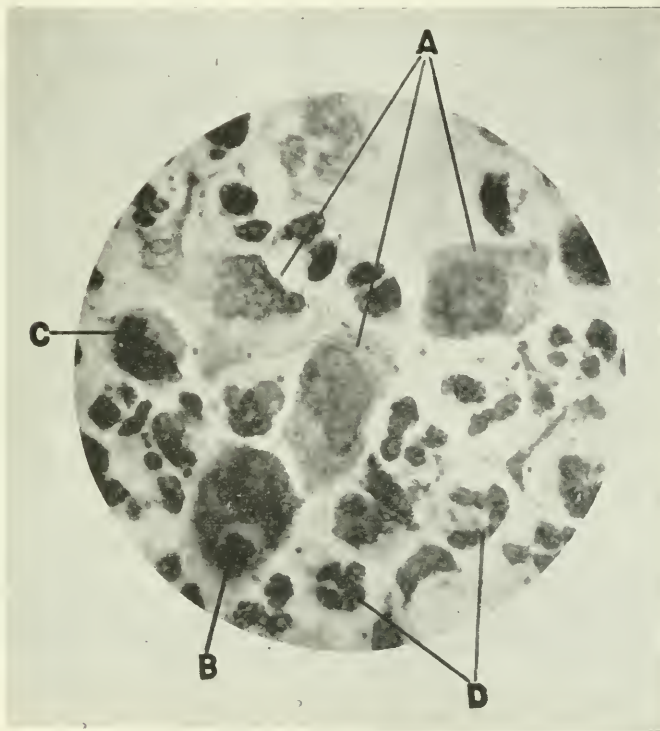


Fig. 27.—Photomicrograph showing types of cells in pathologic cerebrospinal fluid.  $\times 1000$ . A, endothelial cells. B, endothelial cell which has engulfed a polymorphonuclear leucocyte. C, large mononuclear cell. D, polymorphonuclear leucocytes.

chamber is 16 mm. square instead of 9 mm. as in the blood counting chamber, and is 0.2 mm. deep instead of 0.1 mm., as in the blood counting chamber. This chamber, therefore, allows of a smaller error than the blood chamber. In using this chamber the cerebrospinal fluid is drawn up to mark 1 in the leucocyte pipette and the diluting fluid to mark 11. The cells in the whole chamber are counted and the resulting number is multiplied by 11 and divided by 32. As a diluent for the fluid, a solution of methyl violet (methyl violet 0.2 gm. glacial acetic 5 c.c. and water to 100 c.c.) has been found very useful, although ordinary 2 to 3 per cent acetic acid will do to destroy the red cells in the fluid. The Fuchs-Rosenthal chamber, while more accurate,

is not absolutely necessary, an ordinary blood counting chamber will suffice. The technic is as follows:

The methyl violet or other diluent is drawn up to mark 1 in the pipette and the cerebrospinal fluid to mark 11. In order to obtain the number of cells in one cubic millimeter of undiluted fluid, the result obtained is multiplied by 11 and divided by 9. A rougher yet fairly accurate clinical method is to draw up glacial acetic acid in the pipette and blow it out, and then draw up the cerebrospinal fluid. This is sufficient to dissolve the red blood cells. No allowance has to be made, then, for diluting fluid. The cells must be counted immediately after withdrawal from the body, as they undergo autolysis on standing. If it is impossible to count cells in the fluid immediately after its withdrawal from the body, the cells may be preserved by the addition of 2 to 3 drops of a 3 per cent acetic acid solution to 5 c.c. of

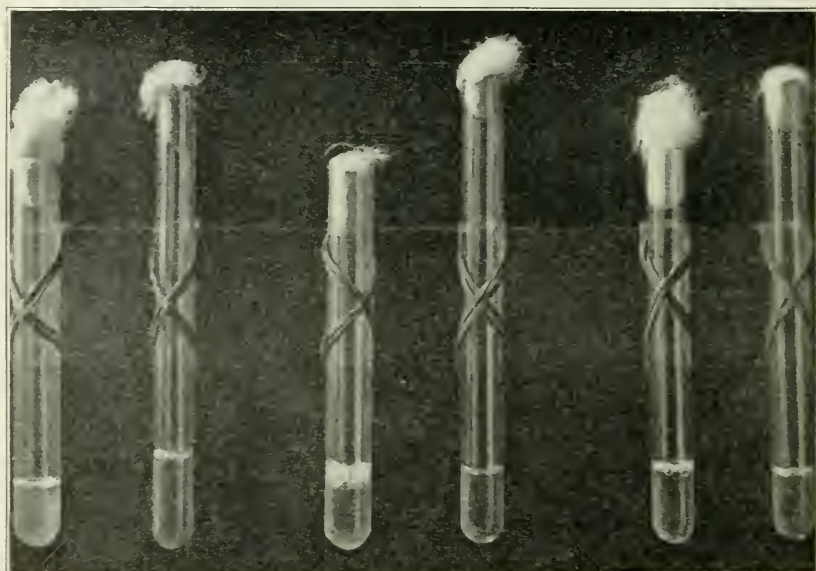


Fig. 28.—Photograph showing agglutination of meningococci by the macroscopic method.

- A. Emulsion of meningococci + 1:10 dilution of antimeningococcus serum.
- B. Control of emulsion of meningococci + salt solution.
- C. Emulsion of meningococci + 1:160 dilution of antimeningococcus serum.
- D. Control.
- E. Emulsion of meningococci + 1:640 dilution of antimeningococcus serum.
- F. Control.

cerebrospinal fluid. The subsequent count will not be very accurate, but will give a good idea of the number of cells in the fluid.

*Differential Cell Count.*—When the fluid is turbid as in suppurative meningitis, a differential cell count may be made on an uncentrifuged fluid. If the fluid is clear or only slightly opalescent, it is best to centrifuge the fluid for from fifteen to thirty minutes at about 1500 revolutions per minute. It should be stained with Wright's stain or methylene blue.

*BACTERIOLOGICAL EXAMINATION.*—In suppurative meningitis where the cerebrospinal fluid is thick, a slide may be prepared from the uncentrifuged fluid. When the fluid is clear, it should be centrifuged for several minutes,

and the sediment examined. Where suppurative organisms are suspected, it is best to stain with both methyl blue and Gram stain. When tubercle bacilli are searched for, the fluid should be allowed to centrifuge at high speed for thirty to sixty minutes and stained by the Ziehl-Neelsen method. If a pellicle is found in the fluid, the pellicle should be stained for tubercle bacilli.

**CULTURE MEDIA.**—Several drops to 1 c.c. of cerebrospinal fluid or its sediment are planted on a culture media, the kind of media depending on the bacteria looked for. In cases suspicious of poliomyelitis anaerobic cultures should be made, as it is claimed that in this way the organism will be found in culture. In most other cases blood agar is the best culture medium. The culture is examined after 24 and 48 hours incubation.

**Agglutination.**—In order to make certain that the antimeningococcus serum used in treating a given case of meningococcus meningitis is specific for

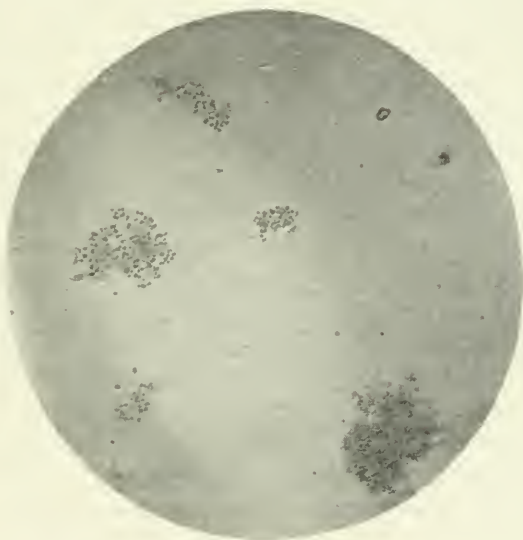


Fig. 29.—Agglutination of meningococci by the microscopic method. (Magnification 800 diameters.)

the particular strain of meningococcus in question, the bacteria obtained by culturing the cerebrospinal fluid of the patient should be tested by the antimeningococcus serum used for treatment. Unless the bacteria are agglutinated by the serum, the given serum is not to be used in the treatment, and a serum must be found that will agglutinate the particular strain of meningococcus in question.

**Technic.**—Two methods have been described for this purpose: the macroscopic and the microscopic.

The macroscopic method is carried out by washing down the growth of the culture in question with two or three c.c. of an 0.8 per cent sterile salt solution, and adding 0.2 c.c. of this emulsion to various dilutions, (1:10, 1:20, 1:40, etc., to 1:2000) of antimeningococcus serum, and incubating it for 15 to 20 hours. If the bacteria in the culture is meningococcus, a floccu-



lent precipitate will appear in the tubes, containing as high a dilution as 1:2000, or even higher. If it is not meningococcus, it will either not agglutinate at all, or it will agglutinate only in very low dilutions, such as 1:50, or 1:10.

The microscopic method consists of using one drop of antimeningococcus serum, one drop of whole human blood in a sodium citrate solution (1 drop of blood to 2 per cent sodium citrate in salt solution), and one drop of a suspension of the organism to be tested. This is mixed in a pipette, heated for 20 minutes, spread on a slide, stained and examined under the microscope. If the bacterium is meningococcus, a distinct clumping will be seen under the microscope. This should be controlled by a suspension of meningococcus with normal horse serum, or by a suspension of some other bacteria with antimeningococcus serum. (Figs. 28 and 29.)

*Guinea Pig Inoculation.*—Five to 10 c.c. of cerebrospinal fluid is injected subcutaneously into the anterior abdominal wall of a normal guinea pig. The needle should be pointed downward and laterally toward the inguinal region. Six weeks later, or sooner in case the guinea pig dies, an autopsy should be performed and the inguinal and mesenteric lymph nodes, as well as the liver and spleen, should be examined for tubercles.

**WASSERMANN REACTION.**—While there are diseases other than syphilis, such as leprosy or sleeping sickness, that give a positive Wassermann reaction, the test is sufficiently reliable to consider the patient syphilitic when the reaction is positive.

The technic of the Wassermann test on cerebrospinal fluid is the same as that on blood, except that the cerebrospinal fluid does not have to be heated as in the case of blood serum; and that the amount of cerebrospinal fluid taken for each test must be greater than the amount of blood used.

Usually 0.2 c.c. of cerebrospinal fluid is taken for each of the tubes set up, varying the amount of antigen added to the tubes. As a control 0.4 c.c. of cerebrospinal fluid without antigen is used. Schottmuller advises the use of different amounts of cerebrospinal fluid in each of the test tubes. This gives a more or less quantitative result as to the degree of positiveness of the fluid.

(To be continued.)

## THE EFFECT OF BUFFER SALTS ON BLOOD COAGULATION\*

BY BENJAMIN JABLONS, M.D., NEW YORK CITY

FOR a long time it has been known that certain salts have the property of retarding coagulation of blood when added to blood in vitro. Some of the more commonly known salts employed for this purpose are the citrate, fluoride and oxalate of either sodium or potassium. The oxalate is supposed to act by virtue of its ability to precipitate the calcium in an insoluble form. This is similarly true of the fluoride. Citrate, on the other hand, although its action is not completely understood, is supposed to produce its anti-coagulating effect through its dispersion of the calcium ion. The effect of buffer salts on the clotting of blood has not been studied, as far as can be gathered from a perusal of the available literature. Some recent experiments carried out on the physical and chemical changes involved in the clotting of blood would indicate that hydrogen-ion concentration plays an important rôle in this process. It was, therefore, thought advisable to study the effect of the addition of various buffer salts on freshly drawn blood, and to compare this effect with the addition of other salts.

To a series of tubes containing respectively sodium chloride, sodium citrate, sodium bicarbonate, sodium taurocholate, monobasic and dibasic potassium phosphate, freshly drawn human blood was added in 1 c.c. amounts. The clotting time was controlled by the addition of 1 c.c. of blood to a clean tube in which no salt had been placed. The effect on the blood can be seen from the appended tables. Blood No. 2 represents a specimen taken from a patient suffering from thromboangiitis obliterans; blood No. 1, from a normal subject. One noticeable feature about the reaction was the varied effect of the various salts on blood coagulation. In the first experiment sodium bicarbonate had been omitted. The blood was allowed to remain at room temperature, so that any possible hemolytic effect might be noticed in the supernatant plasma or serum, after the clot or cells had settled to the bottom of the tube. A striking phenomenon noticed was the inhibition of coagulation produced by the buffer salts. The effect was similar to that which was obtained with sodium citrate. Both of these bloods clotted normally within three minutes after being drawn. In the first instance, the blood drawn from the thromboangiitic patient clotted after ten minutes when added to sodium chloride. This phenomenon also occurred in the tube to which sodium taurocholate had been added. No hemolysis was evident in the control tubes containing the normal blood and blood taken from the thromboangiitis case. The tubes containing sodium chloride in both instances showed hemolysis. The taurocholate tubes in both instances showed very marked hemolysis with production of a soft clot. The tubes containing

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sodium bicarbonate similarly showed marked hemolysis, although the clot failed to show the presence of methemoglobin after twenty-four hours. The tubes containing sodium citrate, while remaining incoagulable, showed definite hemolysis in the supernatant plasma. The tubes to which the buffer salts had been added showed a very interesting phenomenon. In the tubes containing the dibasic potassium phosphate, the blood remained incoagulable for forty-eight hours, and the blood was bright red in color resembling arterial blood or blood containing oxy-hemoglobin. This was evident in both

TABLE I

	2½ MIN.	5 MIN.	7½ MIN.	10 MIN. OVER	
Control containing blood alone 1 c.c.*	±	++	++	++	No change
Sod. Citrate 0.1 gm.					
Blood 1 c.c.	0	0	0	0	
Sod. Chloride 0.1 gm.					
Blood 1 c.c.	0	++	++	++	
Dibasic Pot. Phosphate 0.1 gm.					
Blood 1 c.c.	0	0	0	0	
Monobasic Pot. Phosphate 0.1 gr.					
Blood 1 c.c.	0	±(soft clot)	+(soft clot)	+(soft clot)	
Sodium Bicarbonate 0.1 gr.					
Blood 1 c.c.	++(3 min.)	(+) (soft clot)	+(soft clot)	+(soft clot)	

\*Blood taken from normal patient.

TABLE II

	2½ MIN.	5 MIN.	7½ MIN.	10 MIN.	12½ MIN.	15 MIN.	20 MIN.
Control containing blood alone 1 c.c.*		(coag 5 min.) +	+	+	+	+	+
Sod. Citrate 0.1 gm.							
Blood 1 c.c.	0	0	0	0	0	0	0
Sod. Taurocholate 0.1 gm.							
Blood 1 c.c.	0 (thick)±		+	++	++	++	+
Monobasic Pot. Phosphate 0.1 gm.							
Blood 1 c.c.	0	0	0	0	0	0	0
Dibasic Pot. Phosphate 0.1 gm.							
Blood 1 c.c.	0	0	0	0	0	0	0
Sod. Chloride 0.1 gm.							
Blood 1 c.c.	0	±	+(?)	+(?)	++	++	++

\*(Blood taken fifteen minutes after intravenous injection of buffered citrate solution.)

the normal and pathologic blood. In the tube containing the monobasic phosphate, on the other hand, the blood, while apparently remaining incoagulable, became very dark in a very short period of time as compared with the tube to which the dibasic phosphate had been added. The viscosity of the blood to which monobasic potassium phosphate had been added, seemed similarly increased, the blood failing to flow as freely as in the tube containing the dibasic potassium phosphate. The change in color was very striking, the blood after twenty-four hours having a chocolate hue, whereas the dibasic

phosphated blood had even a lighter hue than when originally drawn (blood was taken from median basilic vein). Hemolysis was only slightly present in the dibasic phosphated blood, whereas it was much more marked in the blood containing the monobasic phosphate.

One sample of blood which had been kept and which was prevented from coagulating by the addition of the buffered citrate solution (sodium citrate, sodium chloride, and buffer salts) failed to show any hemolysis after forty-eight hours.

## FAMILIAL HYPERTENSION WITH REPORT OF A CASE\*

BY JACOB ROSENBLUM, M.D., PH.D., PITTSBURGH, PA.

### I. INTRODUCTION

WHILE it is true that today we know there are certain diseases that are hereditary, it is not generally known that there exists an hereditary type of hypertension.

An explanation<sup>1</sup> of this condition may be offered, if we can accept the "growing point" theory which holds that there are two primary axial points in the early formation of an organism, i.e., positions from which cells and daughter cells extend and developing from these central axial points are formed other secondary or lateral forming points that produce the anlagen of the limbs and internal organs.<sup>2</sup> This theory is accepted by biologists to hold for plants and lower organisms and probably a similar primary condition is present in the higher animals with axial growing points in the pituitary region (superior) and sacral region (inferior) from which lateral growing points extend to reproduce the limbs and internal organs. The pattern of these growing points for the different species is undoubtedly inherited. Abnormalities form from a splitting or cleavage in a growing point and when it is an axial point, there is produced the teratoid monster with duplicate heads and trunks, etc., and when it is one of these secondary growing points there are produced limbs or organs. We know that many of these results of abnormal cleavage are called mutations and are inherited according to Mendelian ratios, for example, polydactylism.

In the plant, the primary growing points are active throughout its existence, whereas in animals they cease to function as such when the anlagen of the brain and the nervous system have been developed; if for some reason they do not cease to function, the tissue is forced through the roof of the mouth forming a teratoma, or if the inferior, a sacral teratoma is produced.

If the secondary growing points escape abnormal cleavage, thus producing duplicate organs (and they of course usually do escape), they develop the anlage of the limbs and organs, which vary in size according to the

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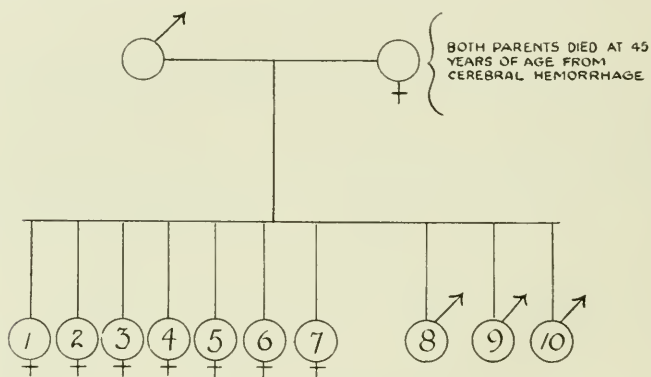


amount of original "vital" or chemical energy released and evidently an organ cannot develop beyond the original possibilities of the anlagen which explains the state termed aplasia or arrest in development, which, after all, is not an arrest in but the extent of development, that is the organ is normal in shape and fully developed according to its possibilities, but with its functional capacity lowered in keeping with the size. Certainly there is proof enough from biological investigation that cellular potentialities are inherited, for this one need go no farther than Morgan's studies on heredity in the fruit fly (*Drosophila Amphylopla*).

I think that the hereditary factors in the case of family hypertension that I am describing, lie somewhere in the potentialities of these secondary growing points.

## II. DESCRIPTION OF CASE

Following is the family tree of case of familial hypertension:



Let us now consider these descendants separately:

1. Died at forty-seven years from cerebral hemorrhage. Had suffered from hypertension.
2. Is forty years and has hypertension.
3. Is forty-four years old and has hypertension.
4. Is forty-six years old. Systolic 290, diastolic 140.
5. Is forty-nine years old. Systolic 220, diastolic 160.
6. Is thirty-five years old. Blood pressure normal. Will she develop hypertension between the ages of forty to forty-five years?
7. Is thirty-three years old. Blood pressure normal. Will she develop hypertension between the ages of forty to forty-five years?
8. Died at forty-seven years. Had hypertension. Systolic 240, diastolic 160.
9. Died at forty-five years of age. Had hypertension.
10. Died at forty-two years of age. Had hypertension. Systolic 240, diastolic 140.

Case four was married to person who died at fifty years of age and who suffered from high blood pressure for ten years previous. They have a

daughter aged twenty under my care who is normal except for constipation. Will she develop hypertension between the ages of forty to forty-five years?

Raymond<sup>3</sup> cites some cases which showed a family fate as regards a disposition to hypertension and cerebral hemorrhage. Albutt<sup>4</sup> states that in respect to arteriosclerosis there are two modes of hereditary transmission, the direct and the indirect; the direct, that form which he has named the decreescent, or primary, which consists apparently either in an original frailty or toxic susceptibility of the arterial structure, a frailty or susceptibility which like other such peculiarities, may run in families; the indirect, that form which he has named the hyperpietic, a secondary event, which apparently depends not upon an original arterial frailty but upon a proclivity to some ill-understood poisoning or perversion making for high blood pressures; and to these high blood pressures it is that the soundest arterial structures, thus subjected to praeternatural stresses will succumb. He also emphasizes the fact that we must separate high blood pressure cases from premature arterial decay.

Ehrmann<sup>5</sup> has lately described a case of familial arteriosclerosis, extending for two generations.

Of the cases that I describe, Cases 4, 5, and 10 have been under my observation for some years, a Jewish family, very active, successful, and of a nervous temperament. They first present symptoms of hypertension but most of the tests for kidney function are normal. After several years the kidney function tests show kidney involvement, and casts and occasional protein is present in the urine. These cases seem to bear out the ideas of Moscheowitz,<sup>6</sup> Albutt, and others, who consider the hypertension a primary disease and the nephritis secondary.

It is certainly true that no one can view the family tree given in this paper without feeling that heredity has produced in this group either a congenital aplasia of the arterial system, or a susceptibility to some endogenous toxic substance whose effect shows itself between the ages of forty to forty-five years.

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## LABORATORY METHODS

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### A PORTABLE IRRIGATION APPARATUS FOR THE TREATMENT OF WOUNDS WITH LIQUID ANTISEPTICS\*

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BY BEVERLY DOUGLAS, M.D.

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ABOUT 24 months ago the writer began a study of wound irrigation with a view of improving methods. Individual open bowls with small hand syringes as commonly used for irrigation seemed to offer many disadvantages from waste of solution by spilling and evaporation and from danger of contamination. From the nurse's standpoint they were not convenient. Individual closed gravity irrigators seemed to involve unnecessary expense both from the high initial cost and from breakage and rubber deterioration.

The portable irrigator herewith described is the result of experiments to simplify and standardize the diverse forms of apparatus employed for the treatment of wounds with antiseptic solutions. We regard it as an improvement over the apparatus of le médecin-major Perret described for war use by Carrell and Dehelly† and consisting of a reservoir of Dakin's solution supported at the proper height on a dressing wagon. The experimental and practical work with it was done with the co-operation and aid of the surgical and nursing staffs of the Johns Hopkins and of the New Haven Hospitals whose valuable assistance is gratefully acknowledged.

#### DESCRIPTION

On account of limits of space we are unable to give more than an outline of construction here. As put upon the ward it is arranged as shown in the drawing.

The *Carrier* has a circular base (1) of rigid lacquered metal with an aluminum deck (2) provided with four circular perforations of sizes to fit the two bottles and two sterile-tip containers. Attached to one of the upright portions of the handle (4) is a pair of adjustable hooks by means of which the apparatus can be suspended from the bed or carriage. The larger container is preferably an opaque bottle of half-gallon capacity which serves as a reservoir. The rubber stopper for it is held in place by a wire strap (7) secured to a metal collar. The smaller container is provided with a gage consisting of a vertically extending series of graduations (16); each graduation preferably indicating an ounce of solution. It is transparent so that the amount delivered from the large container can be

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\*From the Department of Surgery, Yale University.

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†"Infected Wounds"—Carrell and Dehelly, 1917, New York, Paul B. Hoeber Co.

readily determined. The stopper of the larger bottle is secured by a collar and a strap.

It is hardly necessary to go into details of the disposition of the glass and rubber tubing, and the bulb and tip, except to say at (17) a horizontally disposed "L" tip is connected by a small rubber tube to the lower end of the gaging pipe. This tip is flattened at its distal portion so that its aperture may be accurately positioned by raising or lowering the gage pipe (15). Attached to the outer end (15) is a rubber tube of sufficient length to reach conveniently from the head of the bed to the feet of the patient. When not in use it is kept wound upon the reel as shown. Near its end is a small snap thumb clamp (11) with which the discharge of solution may be regulated. Sterile glass dropper tips are provided in one of the small tip

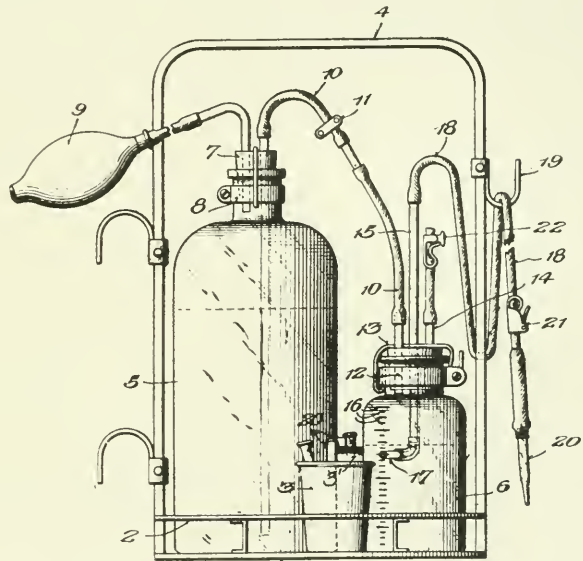


Fig. 1.—A portable irrigation apparatus of extensive application in wound treatment with antiseptic solutions.

containers (3). These are changed after each dressing and the used tip placed in the other small tip container (3)'.

#### METHODS OF IRRIGATION

In operation the large container is filled with Dakin's solution, bichloride or other antiseptic, the clamp between the two bottles is opened and by pressure created in the first bottle by the hand bulb the liquid is forced to flow into the smaller graduated container until the latter is filled to the uppermost graduation. The apparatus is then ready for use either for (1-A) forced feed irrigation, *without measurement* (1-B) forced feed irrigation *with measurements* or for (2) a *gentle siphon feed irrigation*. To accomplish (1-A) the vent pipe (14) is closed by a stopcock and the tip (17) on the lower end of the gage pipe (15) is positioned immediately below the level of the liquid in the small container, then by pressing the bulb, liquid is forced from the



large to the small bottle and from the latter through the gage pipe and tubing (18) to leave through the sterile tip. The level in the container remains constant as long as any solution is left in the large bottle. To accomplish (1-B) the hand bulb (9) is removed from the first bottle and connected with the smaller one through the vent pipe (14) and the screw clamp (11) between the two bottles is closed so as to prevent back flow of solution from the latter to the former. The gage pipe is then lowered to any point desired below the level of the liquid depending on the exact quantity which the operator wishes to inject. This method allows any amount of solution to be expelled from the sterile tip under any desired pressure from the hand bulb. To accomplish (2) the siphonic feed irrigation, which is the usual method employed on the wards, the graduated container is filled as described and the lower end of the gage pipe (15) is positioned at any point on the graduation depending on the amount of solution to be used for particular irrigation. The vent pipe (14) is then opened and the tray suspended above the patient preferably on the bed by means of the hooks. The only precaution necessary is to have the graduated container above the level of the dropper tip when the latter is in position to irrigate the wound. With the apparatus in this position the solution will be discharged from the container by siphonic action until the liquid in it drops below the lower end of the gage pipe. *The bubbles of air which will then rise in the gage pipe (15) will indicate to the operator or attendant that the desired amount of solution has been discharged* so that he may instantly shut off the clamp at the end of the tube and may stop the flow of solution before the action of the siphon is *destroyed by the ingress of air*. Successive irrigations may be accomplished by any one of the three methods by simply changing the glass tip at the end of the outlet tube. Used tips are placed in antiseptic solution in one of the containers. The lightness and portability of the apparatus will be found to facilitate the rapid and sterile handling of the antiseptics during successive irrigations.

#### ADVANTAGES IN USE

1. It offers a closed system and therefore provides against waste, contamination, and deterioration of solution.
2. The combination of a large dark bottle and a similar transparent gage bottle offers a method of preserving a quantity of chemically unstable solution, while at the same time it provides a ready means for measuring and delivering any quantity at any time. This exactness of delivery has been found very economical.
3. The bubble method of visibly gaging the amount of solution delivered is extremely simple and accurate.
4. The use of sterile tips which may be changed easily from patient to patient saves a large quantity of apparatus and does not hazard the sterility of the wound in the least degree.
5. The use of force feed or siphon feed irrigation at will, and the ability to shift instantly from the one to the other has made its application very

extensive for (A) routine ward irrigation by the Carrell-Dakin technic or (B) for Dakin's bichloride, boric or other moist compresses; (C) for cleansing purposes. The operator has found the apparatus very valuable for cleansing exudate from the surface of grafts or of infected wounds. A particularly useful feature of the force feed method is the fact that where a wound occurs on the lower surface of an extremity or of the trunk it may easily be cleaned by a fine stream of the solution under pressure without touching it with gauze.

#### SUMMARY

1. The apparatus employed for the irrigation of wounds with antiseptic solutions in the average hospital is needlessly wasteful and inconvenient, and in certain instances insanitary.

2. The apparatus described obviates these disadvantages and standardizes irrigation treatment. This has been demonstrated in the Johns Hopkins and the New Haven Hospitals.

#### A SIMPLE AIR INTERRUPTER\*

By RAYMOND F. HACKING, RICHMOND, VA.

THERE are a number of interrupters available for use in carrying out artificial respiration on animals in laboratories equipped with means for compressing air, but, in our experience, these have been quite expensive or unsatisfactory in operation. In order to obtain efficient air interruption at a minimum of expense, the following simple apparatus was constructed out of odds and ends in the laboratory.

Briefly, the apparatus consists of a magnet (obtained from an old Harvard Apparatus Co. Vibrating Interrupter) so placed in regard to a lever that the latter may be caused to open and close an automobile pet-cock. The current for the magnet may be derived from four dry cells or, by means of a transformer, from the ordinary alternating lighting current. To make and break the current, a Harvard kymograph and the spring from the Harvard Vibrating Interrupter have been utilized. The drum of the kymograph is covered with paper in the usual manner; and through the paper, small holes are cut at spaces depending on the rate of the drum's revolution and the number of interruptions desired per minute. The magnet is so wired that the current passes through it when the wire tip of the vibrating interrupter spring comes in contact with the exposed metal of the drum; the current being broken when the revolution of the drum causes the paper to be interposed between the wire tip and the drum. It is essential, of course, that the drum's surface be brightly polished, in order to insure good contact. The current being broken, a spring, obtained from a discarded glass syringe.

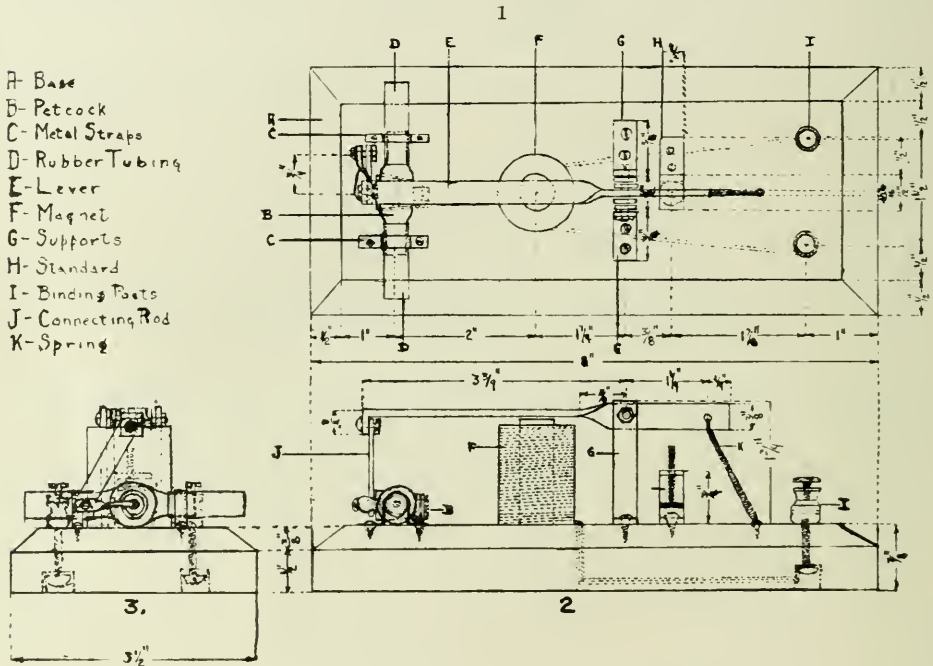
\*From the Laboratory of Pharmacology, Medical College of Virginia, Richmond, Va.  
Received for publication, March 28, 1923.

attached to the end of the lever, draws this end down, thereby closing the valve.

Details of the construction of the apparatus are given in the diagrams, 1, 2, 3; the first giving the appearance as seen from above; the second as seen laterally; while the third shows it as looked at from the end.

The base, A, is a piece of hard wood,  $8 \times 3\frac{1}{2} \times \frac{7}{8}$  inches, the edges being leveled. Holes were bored 1 inch from the edge (I) and counter-sunk from the back for the binding screws.

The lever (E) was made from a piece of soft flat steel. Its dimensions were  $5\frac{5}{8}$  inches long;  $\frac{3}{8}$  inch wide and;  $\frac{1}{16}$  inch thick. Three holes,  $\frac{1}{8}$  inch in diameter, were bored through it;— $\frac{1}{4}$  inch from one end;  $1\frac{1}{4}$  inch from



Figs. 1, 2, 3.

the first hole; and  $\frac{3}{16}$  from the opposite end. Next, the end of the lever containing the two closely placed holes was placed for two inches of its length between the jaws of a vice, and, by means of a wrench, the remainder of the lever was twisted at right angles to the part in the vice. Finally,  $\frac{3}{8}$  inch of the longer end of the lever was bent at a right angle.

G, G, represent two supports, made from soft steel similar to that used for the lever ( $\frac{3}{8} \times \frac{1}{16}$ ). The length of each is  $2\frac{1}{2}$  inches,  $\frac{3}{4}$  inch of each being bent at right angles and drilled to receive small wood screws. A hole was drilled  $\frac{1}{4}$  inch from the longer end; and a second hole in the center; these holes being  $\frac{1}{8}$  inch in diameter.

The standard (H) was made from similar steel and is  $1\frac{3}{4}$  inches long. One-half inch at each end was bent at right angles, the directions being opposite. Two holes were bored in one of the bent pieces, in order that

it would serve at the base while one hole was bored in the other bent end, this hole being tapped to receive a long machine screw.

A connecting rod (J) was made from steel similar to that used in the other metal parts. The length of this rod depends on the type of valve selected. This rod serves the purpose of connecting the end of the lever (E) with the valve stem.

The valve (B) is an automobile pet-cock. It is essential that it work with little friction; to ensure this, the spring was removed and suitable metal washers substituted. The arm of the valve stem was turned at right angles and a hole,  $\frac{1}{8}$  inch in diameter was bored  $\frac{3}{4}$  inches from the center.

The metal straps (C, C') were made from thin sheet iron, bent to conform to the shape of the valve.

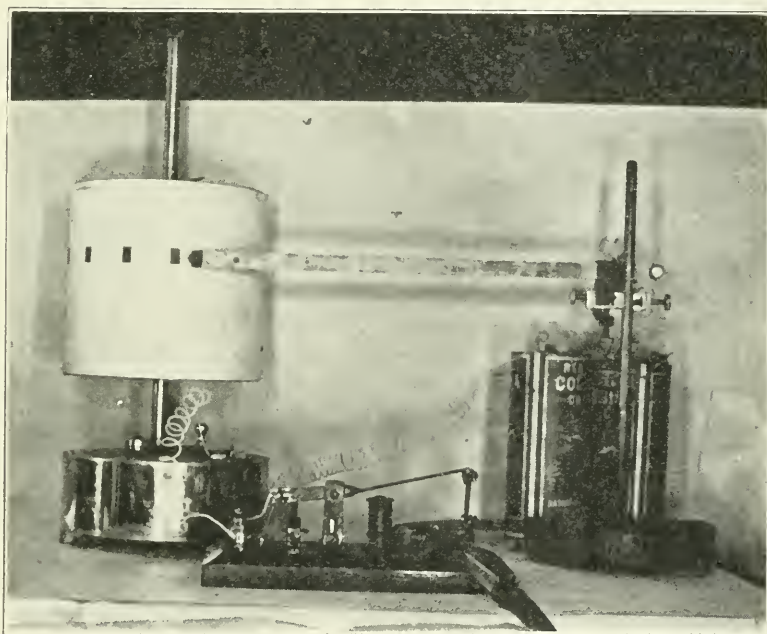


Fig. 4.

The magnet (F) is placed in position, and holes bored through the wooden base to allow the wires to pass to the binding posts (I, I).

Small machine screws were used to connect the valve stem and connecting rod and lever and connecting rod. A long machine screw served as shaft for the lever. The lever was centered between its supports by thin metal washers. The various bearings should be tight enough to eliminate lost motion as much as possible but not cause friction too great for the magnet or the light spring to overcome. To prevent or lessen air leakage, it is desirable to lubricate the valve with a heavy oil. A fairly powerful magnet is necessary; the one obtained from the Harvard Vibrating Interrupter was found very satisfactory. It should not be more than  $1\frac{1}{2}$  inches high.



The photograph represents the apparatus as set up for use; dry cells being used as the source of the current. Should it be desirable, several rows of holes may be cut in the paper; by this and using a heavily weighted fan, almost any respiratory rate can be obtained; while the relation between inspiration and expiration can be determined by the relative size of the holes and of the intervening paper. It is, of course, immaterial which opening of the pet-cock be used for intake or outlet of the air (D. D).

## THE STANDARD WASSERMANN REACTION\*

BY REUBEN OTTENBERG, M.D., AND IDA WISLER, NEW YORK

THE standard Wassermann technic proposed by Kolmer† as the result of a long series of careful studies deserves thorough trial.

We have compared the Kolmer test with the test as usually carried out by us in 204 cases. The number is not large but the work has given considerable information because the tests were carried out on selected cases belonging exactly to the group in which a refined and standardized technic is necessary—namely the weak positive cases;—almost all were cases under treatment.‡

In the first 62 cases tested, we found the Kolmer test a great deal weaker than the Wassermann test as performed by us.§ We felt that perhaps we were not carrying out the test exactly as Kolmer desired. We therefore went to Dr. Kolmer's Laboratories in Philadelphia where we picked up a number of finer points of technic which are worth describing. Thus we learned that sodium chloride in the form of tablets for normal salt solution (Parke, Davis & Co.) was not satisfactory. It interfered to some extent with hemolysis (probably due to other substances than NaCl present in the tablet). Merk's C.P. NaCl was better. We found that we had been using too much complement. In a titration where two hazy looking tubes preceded a tube that was completely hemolyzed, one could safely choose as "one full unit," the tube directly before the completely hemolyzed one. We were told that one could take final readings on the daily titrations at the end of 40 to 45 minutes instead of waiting the full hour.

Also, in comparison to the 2 per cent cell emulsion used in Kolmer's laboratory, the 2 per cent cell emulsion that we had been using was distinctly

\*Received for publication, March 22, 1923.

†Kolmer, *The Jour. of the Am. Med. Assn.*, Sept. 3, 1921, lxxvii, 776-778. Also series of thirty-two papers published beginning in the *Am. Jour. of Syph.*, Jan. 1, 1919.

‡We are indebted for many of the cases obtained from the Vanderbilt Clinic to Dr. J. G. Hopkins.

We are indebted to Dr. Kolmer for the antigen used.

§Ottenberg, *Methods of Determining the Optimal Amount of "Antigen" for the Wassermann Reaction*, *Journal of Immunology*, ii, No. 1, Dec., 1916.

Correct and Incorrect Methods of Performing the "Daily Titrations" for the Wassermann Reaction and Other Forms of Complement Fixation. *Journal of Immunology*, ii, No. 1, Dec., 1916.

On the Reliability of the Wassermann Reaction, *Archives of Internal Medicine*, March, 1917.

weaker. Therefore, when we again took up the work we always at the last washing of our sheep cells, centrifuged for 25 minutes instead of 10 minutes in order to get the same concentration of cells as Dr. Kolmer was using.

It was about this time also that Dr. Kolmer announced the fact that he was changing his dosage of patient's serum by decreasing his amounts more gradually.

All these points we applied to the remaining 142 cases that were tested out. These yielded much more satisfactory results, and on these cases we shall report.

Our average complement titre agreed very well with that of Dr. Kolmer. We tried the use of 1, and  $1\frac{1}{2}$  units of complement along with the regular 2 full units of complement prescribed by Kolmer. While better fixation was obtained in many instances, more or less anticomplementary effects resulted in others. We therefore adhered to the use of 2 full units of complement throughout. The quantities of the ingredients for the Kolmer test correspond to half the quantities of the original Wassermann test in a total volume of 3.0 c.c., and the sheep cells are a 2 per cent emulsion.

Each Kolmer test was checked up by a Wassermann test using alcoholic antigen, and 4 hours ice box incubation, and by a Wassermann test using cholesterinated antigen and 1 hour at  $38^{\circ}$  C. incubation. Both tests were the Citron modification used by us as a routine for the last seven years.

The following are the results:

TABLE I

NUMBER OF CASES	KIND OF CASES	KOLMER'S RESULTS	R. O. ALC.	RESULTS CHOLEST.	REMARKS
123	Known syphilis	+++ or ++++	+++ or ++++	+++ or ++++	Agreement excellent
5	Negative	neg.	neg.	+ or ++	Agreement excellent
1	Treated case	+++	neg.	neg.	Kolmer's test detected 1 case
1	Inherited syphilis No symptoms	neg.	neg.	+++	This same result obtained on three different occasions with this patient.
12*	Treated cases	neg.	+ to ++++	+ to ++++	*Seven out of these 12 cases Kolmer missed entirely due to presence of natural amboceptor.
142					

## CONCLUSIONS

The Kolmer test is a good test and might safely be adopted as a standard. There was excellent agreement in the majority of 142 cases.

## ON THE OTHER HAND

The Kolmer test is a rather elaborate two-day test. It uses up much more materials than the Wassermann test. We did not find the Kolmer test more sensitive or reliable than the Wassermann test carefully done according to the technic adopted by us seven years ago. There was a small but definite number (12 cases, 8 per cent) of treated cases which the Kolmer test did not detect at all—cases ranging from doubtful to strongly positive. In many instances this was due to disregard of natural antisheep amboceptor.

On the whole we believe that Kolmer's work offers the best basis yet proposed for a standard technic, but it will have to be somewhat shortened and some way will have to be introduced for dealing with natural antisheep hemolysin.

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THE KOTTMANN REACTION FOR THYROID ACTIVITY. CARBON DIOXIDE IN THE TESTED SERUM\*

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BY STERNE MORSE, M.D., AND CLYDE M. FITCH, M.D., COLUMBUS, OHIO

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SINCE the original publications by Kottmann,<sup>1,2</sup> in 1920 and the appearance of several articles in this country by Petersen, H'Doubler, Levinson and Laibe in 1922,<sup>3,4</sup> considerable interest has been aroused concerning the possibilities of the Kottmann Reaction. In these papers the chief interest has centered around clinical results, which have been very gratifying to these workers. One of the main problems in the work, so far, has been to determine just what relation this reaction may or may not bear to thyrotoxicosis. To date we have performed over 195 Kottmann reactions on known and suspected cases of goitre.

The technic employed is the same as that employed by the originator<sup>1</sup> of the reaction. The principle is based on a well-known phenomenon observed in photography. When silver nitrate is added to potassium bromide a precipitate of silver bromide is formed and if a colloid such as gelatin is present this suspension is so fine as to be colloidal. The photochemical activity of this colloidal silver bromide seems to depend upon the size of the particles of silver bromide in the gelatin. After the gelatin plate or film is allowed to ripen the colloidal particles become larger and also more photosensitive. When a protective colloid is present the coagulation of the silver bromide particles is retarded, and the emulsion consequently remains less photosensitive. In the Kottmann Reaction, serum is substituted for the gelatin and silver iodide for silver bromide, and it is supposed that a silver iodide suspension takes place in the patient's serum. The rapidity of the reaction is supposed to depend upon the amount of protective power of the serum being used in each test.

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\*From the Laboratory of Grant Hospital, Columbus, Ohio.

Read before the Columbus Academy of Medicine, November 27, 1922.

Received for publication, March 12, 1923.

The test is performed in a dark room with a red light for illumination. To 1 c.c. of the patient's serum (freshly drawn) is added 0.25 c.c. of a 0.5 per cent solution of potassium iodide and 0.3 c.c. of a 0.5 per cent solution of silver nitrate, thus making a silver iodide suspension in the serum. Reagents in the reaction are prepared fresh and measured accurately. After a slight shaking to insure thorough mixing the serum is exposed for five minutes to

TABLE I

11-17-22	2½ MIN.	5 MIN.	10 MIN.	15 MIN.	20 MIN.	30 MIN.	40 MIN.	1 HOUR
Mrs. T.—								
Nonaerated	O	O	F	L	L	L	L	Incomplete
Aerated 15 min.	F	L	L	L	L	L	L	Incomplete
Mrs. P.—								
Nonaerated	O	O	O	F	L	L	Unchanged	
Aerated blowing with mouth	O	O	F	F	F	F	L	Incomplete
Mrs. D.*.V.—								
Nonaerated	O	F	L	L	L	L	D	
Aerated 10 min.	L	D	D	D	D	D	D	
Mrs. P.—								
Nonaerated	O	O	O	F	L	L	Incomplete	
Aerated 5 min.	O	F	F	L	L	L	Incomplete	

TABLE II

11-18-22	2½ MIN.	5 MIN.	10 MIN.	15 MIN.	20 MIN.	30 MIN.	40 MIN.	1 HOUR
Mrs. W.—								
Nonaerated	O	F	L	D	D	D	D	
Shaken violent	F	L	D	D	D	D	D	
Aerated 5 min.	O	F	F	F	F	L	L	
10 min.	L	D	D	D	D	D	D	
Mrs. B.—								
Nonaerated	O	L	D	D	D	D	D	
Aerated 10 min.	O	F	L	L	L	L	L	
Mrs. G.—								
Nonaerated	O	O	O	F	L	L	L	
Aerated 15 min.	F	L	D	D	D	D	D	

TABLE III

11-19-22	2½ MIN.	5 MIN.	10 MIN.	15 MIN.	20 MIN.	30 MIN.	40 MIN.	1 HOUR
Mrs. H.—								
Nonaerated	O	O	O	F	F	L	L	
Aerated 10 min.	O	O	F	L	L	L	L	
15 min.	O	F	L	L	D	D	D	

a 500 watt Mazda lamp at a distance of 25 cm. Five-tenths c.c. of a 0.25 per cent solution of hydroquinon is then added. After this the color changes are noted at regular intervals. These changes range from a light yellow to a dark brown or black in some instances.

In this paper it is not our purpose to take up in any way the clinical interpretation of the Kottmann Reaction. Our observations have to do with the collection and handling of the specimen before the reaction is done and during its performance, and have both a practical and theoretical bearing. Soon after starting the tests we were able to easily verify an observation of previous



workers as to the marked variation in the reaction of a specimen of serum if preserved for any length of time, even though the interval be small and the serum be preserved in the ice box. Preservation has a tendency to accelerate the appearance of the color changes. Our policy has been to run tests as soon as possible after obtaining the specimens, and to keep our containers stoppered. Furthermore it was observed that after the addition of hydroquinon, the color change in the serums appeared around the top of the serums first, and was not uniform throughout the rest of the serum in each tube. In cases where retardation was slow there would often be a distinct ring of more pronounced darkening at the surface. This suggested the diminishing of the carbon dioxide content of the serum as a possible causative agent for this beginning change at the surface. This was based on the experience of one of us regarding the important relationship of carbon dioxide to photochemical reactions in the presence of albuminous substances. Abstraction of carbon dioxide causes a relatively important change in the acidity of the serum, and such a change is a fundamentally important variable in controlling the balance of chemical reactions, which is at the bottom of photochemical sensitivity. With this suggestion in mind the following experiments were carried out.

TABLE IV

11-20-22	2½ MIN.	5 MIN.	10 MIN.	15 MIN.	20 MIN.	30 MIN.	40 MIN.	1 HOUR
Mr. R.—								
Nonaerated	O	O	L	L	L	L		
Aerated 30 min.	D	D	D	D	D	D		
15 min.	D	D	D	D	D	D		
10 min.	F	L	L	D	D	D		
5 min.	F	L	L	D	D	D		
Nonaerated	O	O	L	L	L	L		

O—no change of color. F—faint brown color. L—light brown color. D—dark brown color.

Freshly drawn serum was obtained in all cases. The serum was then divided into several equal parts. Into one tube 1 c.c. of serum was placed and the tube stoppered—this specimen was not aerated. At the same time 1 c.c. quantities of the serum were placed in other similar tubes for aeration. Tubes to be aerated were clamped nearly horizontally, thus allowing the serum to present as large a surface as possible. Into each tube was inserted glass tubing extending nearly to the bottom of each tube, which in turn was connected to a suction pump, thus allowing a free suction of air across the exposed surface of the serum. Aeration of different serums in this manner at different lengths of time gave results of which the following are typical:

From these tables it will be noted that variations of the reaction can occur if aeration of the serum occurs in any way. Such might happen, for instance, by the simple exposure to air while the specimen is being centrifuged. A question immediately arises—Is this variation of reaction due to a loss of carbon dioxide, the most likely supposition, or could it be due to oxidation or exposure to light or some other unknown mechanism? To further decide as to the causative agent, the following experiment was performed:

A sample of freshly drawn serum was divided into unequal portions. The smaller (1 c.c.) portion was preserved in a stoppered tube for a few minutes until the larger portion could be thoroughly aerated in a rotated separatory funnel. After five minutes of such aeration 1 c.c. of serum was withdrawn for test and the atmosphere within the funnel was replaced by alveolar air from the operator's lungs. This was once renewed after the funnel had been rotated two and one-half minutes. The serum, in this manner, was again brought into approximately the same carbon dioxide equilibrium in which it had been within the body. The three specimens thus obtained were then simultaneously tested in the usual manner. Table V gives the results of this test:

TABLE V

Miss L.—	2½ MIN.	5 MIN.	10 MIN.	15 MIN.	20 MIN.	30 MIN.	1 HOUR	3 HOURS
Nonaerated	O	O	O	O	O	O	O	F
Aerated 5 min.	F	F	L	L	L	L	D	D
Resaturated	O	O	O	O	O	O	O	O

O—no change. F—faint brown. L—light brown. D—dark brown.

Note:—At the end of twenty-four hours complete reduction had taken place in the non-aerated and aerated specimens, but the resaturated one showed only partial reduction.

Certainly from these experiments it appears that the carbon dioxide content of the serum is playing a very important rôle in our readings of the Kottmann Reaction. Considering the fact that in most instances, decrease in the carbon dioxide content in the serum produces acceleration of color change and in other cases retardation results, it is probable that a still more fundamental variable, possibly hydrogen-ion concentration, is concerned. It should be noted also, that inasmuch as this particular source of error which we have found, has not heretofore been taken account of and as it affects in our experiments, the results in all cases; that all previous work with the Kottmann Reaction must be revised, fundamentally, from this standpoint. So important are small changes in the carbon dioxide content of the serum, that we must exercise great care in the application of the tourniquet, shortening as much as possible the duration of time in which it is applied before obtaining the specimen, thus avoiding any overaccumulation of carbon dioxide in the venous blood. In the future one of two methods will have to be followed—we will have to collect our blood samples under oil with all other precautions customarily employed in obtaining samples for the estimation of carbon dioxide content of blood plasma; or we must devise some method whereby the carbon dioxide error can be eliminated by a standard aeration of all blood serums.

## LITERATURE

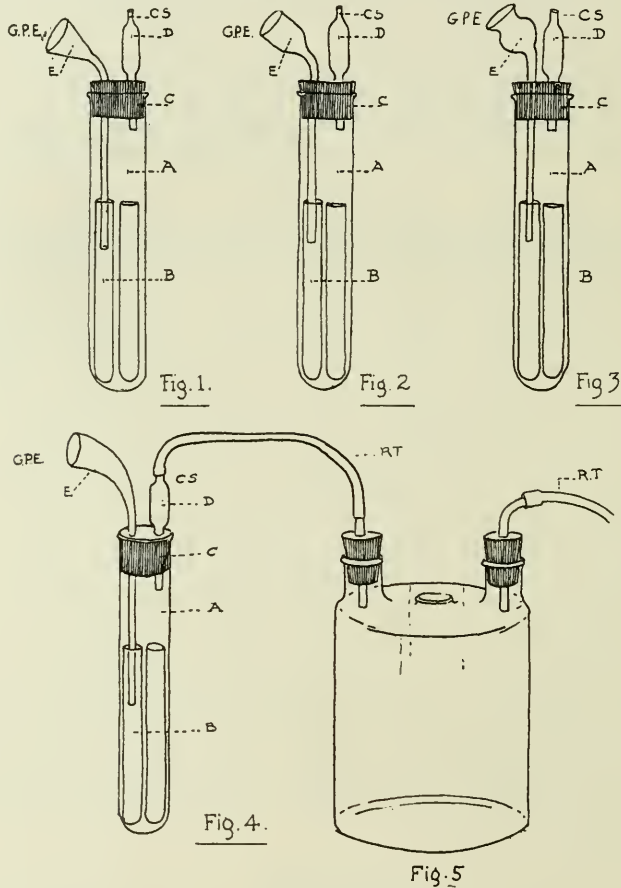
- <sup>1</sup>Kottmann K.: Kolloidchemische Untersuchungen über Schilddrüsenprobleme Schweizerische Medizinische Wochenschrift, No. 30, pp. 644-51, July, 1920.
- <sup>2</sup>Kottmann, K.: Beziehungen zwischen Blut und Organen, Schweizerische Medizinische Wochenschrift, No. 47, pp. 1060-64, Nov., 1920.
- <sup>3</sup>Petersen, W. F., H'Doubler, F. T., Levinson, S. A., and Laibe, J. E.: The Kottmann Reaction for Thyroid Activity. Jour. Am. Med. Assn., lxxviii, pp. 1022-23, April 8, 1922.
- <sup>4</sup>Petersen, W. F., H'Doubler, F. T., Levinson, S. A., and Laibe, J. E.: Studies in the Kottmann Reaction for Thyroid Activity. Arch. of Int. Med., xxx, pp. 386-396, Sept., 1922.

# A SIMPLE METHOD FOR OBTAINING GUINEA PIGS' BLOOD FOR COMPLEMENT\*

BY B. S. LEVINE, PH.D., WAUKESHA, WIS.

*Director of the Clinical Laboratory, U. S. V. Hospital No. 37, (N. P.) Waukesha, Wisconsin.*

IN obtaining blood from guinea pigs for complement used in the Wassermann reaction, it is desirable to avoid the use of anesthetics, since it is maintained by many serologists that anesthetics impair the complement quality of the blood serum. It is likewise desirable to reduce or, if possible, to



eliminate entirely the mortality of the animals, caused by improper puncture or by excessive use of the anesthetic. This becomes especially desirable when one finds animals whose blood possesses a high complement titre.

Drawing blood from laboratory animals is frequently assigned to tech-

\*Received for publication, March 5, 1923.

nical assistants whose operating skill is not always the best. Hence, the necessity for a method possessing the elements of simplicity, reliability, and safety arises. A method based on the principle of suction fulfills all these requirements, and is now in use in our laboratory with success.

Figs. 1, 2, 3, 4, 5 and 6 are schematic representations of the possible arrangements that can be used and in their simplicity are self-explanatory. The parts which are described below are obtainable in every diagnostic laboratory, and the combination can be set up in fifteen to thirty minutes.

- (A) is a large heavy-walled pyrex glass tube.
- (B) are Wassermann tubes into which the blood is collected.
- (C) is a two-hole rubber stopper.
- (D) is a connecting tube of glass.
- (E) in Fig. 1 is made of a small-sized funnel.
  - in Fig. 2 is made of a part of a 75 c.c. chemical pipette.
  - in Fig. 3 is made of a thistle tube.
  - in Fig. 4 is made of a drawn out heavy-walled medium-sized pyrex glass tube, and if bent as shown in the Figure is the best for the purpose.

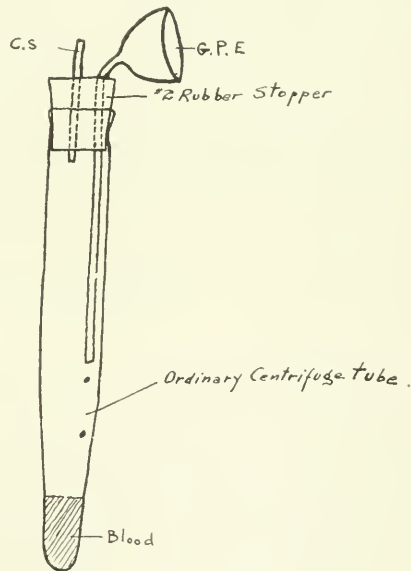


Fig. 6.

In obtaining the blood, suction is applied to the end marked C.S. through the Woulff flask, as is shown in the combination of Figs. 4 and 5. The ear of the animal is then washed, a small incision made, and the end of the glass arrangement marked G.P.E. is placed tightly over it. As soon as the skin of the animal seals the opening and a sufficient vacuum is created, the blood begins to flow from the incision into tube B. When a sufficient quantity of blood is drawn from one animal, another, or a third, and a fourth may be used. Two or three Wassermann tubes may be placed inside the large tube



A when a large quantity of serum is required. If some of the clotted blood collects at the end marked G.P.E., it is only necessary to push it with a clean glass rod into the neck wherefrom it will be carried down into the tube B by the suction. Figure 6 shows a still further simplified arrangement, that can be used with even greater ease than any of the above described. The drawing is self-explanatory.

This method of obtaining blood from the guinea pig requires no skill, and can be used successfully even by a beginner. It is safe, for the use of anesthetics and the necessity to "puncture in the dark" are completely eliminated.

Acknowledgment is here made to Dr. Grill of the Marquette Medical School, Department of Pathology and Bacteriology.

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## EDITORIALS

### *Life and Problems Under a Medical Utopia\**

UNDER the above title, Carr in satire, has portrayed what we are coming to when the world is controlled by the doctors. All, of both sexes, on reaching the marriageable age will undergo strict and thorough medical examination. This will include not only the life-history of the individual but his or her ancestry so far as it can be traced. Ultimately, it will come about that every one will have a full pedigree from the time of the establishment of the Utopia. Those who are found fit will be given certificates, licensing them to marry, or at least to propagate their kind. Whether legal marriage will be required in Utopia is a matter left somewhat indefinite. All who cannot pass the examination will be sterilized so that they cannot beget children either in or outside of matrimony. The young couple, having passed these preliminary medical ordeals, will enter the marriage state. As soon as the wife becomes pregnant she will notify the proper authorities and she will be compelled to submit to an examination as frequently as the

\*London Lancet, May 19, 1923.

medical officer may determine. The expectant mother will be relieved of all burdens in the way of work for a certain period before confinement. When the day set by the medical officer for confinement arrives the woman will go to a lying-in hospital. Artificial labor will be induced under proper aseptic precautions. The birth of the child will be duly registered and the mother before she leaves the hospital will be instructed in all particulars concerning breast-feeding. She will be required to take the infant every week to the hospital or dispensary in order that it may be weighed, its progress carefully watched, and its feeding superintended. When the child reaches the school age, all these procedures will be turned over to the school authorities. After her first confinement the mother will be carefully examined and notified whether or not she will be permitted to bear another child. How succeeding pregnancies are to be avoided is left somewhat uncertain. The couple may be advised to practice absolute and continuous continence. The author says that some advise this, but they are usually elderly folk who have forgotten that they ever had sex instincts or worthy ladies who perhaps never developed them or ecclesiastically minded celibates who are apparently anxious to make vicarious atonement for their own infertility by encouraging the highest possible fecundity in others. As a substitute for absolute continence various mechanical preventives or contraceptives may be furnished the married couple.

When the child reaches a certain age it will be carried to the surgeon, and if a boy will be circumcised, while in both sexes the appendix will be removed. The author is quite certain that at some specific age the entire large intestine will be removed from all. He justifies this procedure by high authority. He cites Metchnikoff, who wrote: "It is no longer rash to say that not only the rudimentary appendix and the cecum but the whole of the large intestine are superfluous and that their removal would be attended with happy results." Dr. Barclay Smith writes: "The statement is perhaps a bold one, but I am convinced that the large intestine is a practically useless incumbrance to man." Every year this opinion apparently grows and recently Sir Arbuthnot Lane informs us that he has performed, with complete success, colectomy in early childhood. "Since it has been discovered that the large intestine is a cesspool, certainly everybody should get rid of the cesspool which he carries around with him, as well as that which he formerly built in his back yard."

The next thing is to attend to the teeth. Since recent experiences have shown that pyorrhea is a constant menace to the health and a frequent cause of ill health, all the teeth of the second set should be drawn soon after their appearance and artificial ivories substituted. Of course, every child some time early in life will be not only vaccinated, but treated with a multivalent extract of all the organs in the body. The eyesight will be looked after and spectacles will be in great demand. The most serious thing, however, will be to follow the advice of Freud and practice psychotherapy upon all. Carr says, how marvelous, for instance, to discover, owing to a patient getting blocked on the simple word "long" that all her troubles were due to

her "longing" for a certain young man, whose offer of marriage she had rejected in haste, afterwards repenting at leisure of her refusal! How instructive to read that a young man who has been stammering for several years is easily and perfectly cured when it is realized that his difficulty in articulation was worse for words commencing with "K" and that this was the first letter of the name of a former sweetheart who had jilted him in favor of another man! Indeed, in Utopia the demand for psychotherapy will be unlimited.

Carr's description of how venereal diseases are to be handled is exceedingly interesting. Freudian teaching indicates that if we suppress our sexual desires we are going to suffer from this action sooner or later. It is highly desirable, therefore, that all sexual enticements should be gratified. Every young man will be required to carry with him constantly a prophylactic packet. It is true that he will be advised to practice continence, but it will be recognized that the sexual appetite is strong and that any young man may meet with temptations which he cannot resist, or at least he will not.

After giving us several pages of interesting satire the author drops into common sense and writes: "Probably the greatest danger under a medical autocracy would be that of loss of freedom. A distinguished ecclesiastic once caused quite a grave scandal by saying that he would rather see England free than England sober. I think he was right, and I believe it would be equally correct to say that we would rather see England free than England perfectly healthy. A despotism may be theoretically the best form of government if the ideal despot can be found, but he never can be, for human nature is so constituted that the mere fact of entrusting absolute power to an individual or a group of individuals soon renders them unfit to exercise that power over their fellow creatures. A medical despotism would be no exception to the rule. It is no sufficient answer to say that medical government would be necessarily in the best interests of the governed. A man's conception of what is best for his fellows may be absolutely disinterested and honest, his intentions may be wise and unselfish, but his conclusions and decisions may be erroneous, although the more convinced he is that he is doing right and that he is actuated by the highest and noblest principles, the more likely is he to become a tyrant. The exercise of uncontrolled power is almost always a cause of demoralization, and conscientiousness has to account for some of the darkest chapters of human history."

We have no fear that medical despotism, as portrayed in satire by Carr, will ever prevail on earth. Even if invested with absolute autocracy the medical profession is too sensible to lead the world so far astray.

—V. C. V.



### *A Transfer to Younger Shoulders*

VOLUME VIII of this Journal will soon reach its close. With the September number I am turning over the editorship to Dr. Warren T. Vaughan, Professional Building, Richmond, Virginia. I am herewith asking that after the publication of this notice contributors direct all communications to the above address.

The Journal has acquired a worthy standing, and I do not think I am overstating the matter when I say that it has become the standard publication for laboratory methods and laboratory research along medical lines in this country. However, the Journal speaks for itself and I am willing that my labors as editor-in-chief shall be judged by their fruits. I have reached the period in life when I wish to retire, in part at least, from work. It gives me great pleasure to testify to the cordial relations that have existed and continue to exist between myself and my associate editors, my contributors, and the publishers. The last-mentioned have done everything in their power to make the publication creditable alike to themselves, their contributors, and the profession. My best wishes are for continued success, and I hope from time to time to be able to contribute to the Journal.

—V. C. V.

### *BOOK REVIEWS*

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

#### *Jordan's Bacteriology\**

ORIGINALLY the outgrowth of lectures given to students in the University of Chicago, this book has developed into a textbook of bacteriology standard throughout the country. The general arrangement of the book is not changed from that of previous editions. The chapters on Influenza and Anaërobes have been entirely rewritten and the chapters on Streptococci, Pneumococci and Typhus Fever extensively revised. Additions have been made under the subjects of Immunity, Yellow Fever, etc. The chapter on the methods of study of bacteria has been rewritten and considerably enlarged. The author feels that we must provisionally accept leptospira icteroides as the cause of yellow fever. Concerning influenza, he remarks that the most promising work is that of Olitsky and Gates. Under the anaërobes, he discusses the newer work on botulism.

The author adopts the recent classification of the meningococcus, micrococcus catarrhalis and gonococcus, under the genus "Neisseria."

\*General Bacteriology. A textbook by Edwin O. Jordan, Ph.D., Professor of Bacteriology in the University of Chicago and in the Rush Medical College. Seventh edition. Thoroughly revised. Cloth. Price \$5.00. Pp. 744. Fully illustrated. Philadelphia and London. W. B. Saunders Company, 1922.

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# *The Journal of Laboratory and Clinical Medicine*

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## ORIGINAL ARTICLES

### THE EVOLUTION OF CLINICAL PATHOLOGY\*

BY HARRY GAUSS, M.S., M.D., DENVER, COLO.

TO crystallize our theme we will arbitrarily define clinical pathology as that branch of medicine which seeks to correlate anatomic alterations with clinical symptoms by the refined methods of pathology, serology, bacteriology and chemistry. It follows almost as a corollary that the clinical pathologist functions within the practice of medicine in the diagnosis of disease rather than in the therapeutics of it. However, under certain extenuating circumstances the sphere of the clinical pathologist enlarges and he may supervise the management of certain conditions especially the infectious and contagious diseases where the diagnosis and control of the diseases is made largely by laboratory methods. Such a contingency arose during the World War when the Department of Laboratories and Infectious Diseases was organized in the American Expeditionary Force to handle the special situation.

Under ordinary existing conditions, however, the clinical pathologist operates in his laboratory where he combines the technical information of a multiplicity of sciences, or rather he appropriates such parts of the several sciences as he needs and combines them under the title of Clinical Pathology. While the several sciences he utilizes are regarded as distinct, if we are to believe the curriculums of the schools, we have only to trace them back a comparatively short time to find them originating in a common progenitor, the philosophy of the Middle Ages, and if traced a few centuries further back, the entire subject is lost in the religion and mysticism of the earlier races where its inception took place.

This attempt at the consolidation of miscellaneous portions of a group

\*Read at the Second Annual Meeting of The American Society of Clinical Pathologists, San Francisco, June 26, 1923.

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of sciences which originated from a common source is comparable to the efforts of some of our contemporaries in allied fields of endeavor. The modern physicist sees a merging of all sciences into a universal science. Physics, chemistry, biology and geology will cease to be independent sciences, lose their identities and adopt a common set of principles.

Modern science moves so rapidly that most of us are disturbed to find that the basic principles of the more exact sciences are not nearly so stable as it would be convenient to have them. The chemical element which only a short time ago, we learned, was the beginning of all inorganic and organic structures is no longer regarded as elemental; rather it is considered an intermediate state of organized energy. Even the molecule and atom into which they were split up are still further resolved into electrons and protons, two types of particles, both invisible but independently observable by certain effects they produce. Stars are born and die, much the same as organic life and the transmutation of metals is conceivable. The very elements are born and die, although their life cycle may be thousands or millions of years. There is nothing stable in the universe. Everything is in a state of transition or evolution, and every phenomenon must ultimately be explained in terms of a matter which is granular in structure and electrical in character (Mills). Emerson, the American essayist, must have contemplated a constantly changing universe when he wrote, "the gases gather to the solid firmament; the chemic lump arrives at the plant, and grows; arrives at the quadruped, and walks; arrives at the man, and thinks." So when we find the science of clinical pathology in an evolutionary phase we recognize that this is in accord with all natural law.

Clinical pathology may be said to have been born in May 22, 1922, at St. Louis, Mo., where the American Society of Clinical Pathologists was organized. It is true that clinical pathology existed before this time, but its consideration prior to this date must come under the heading of its embryology. However, since it is our purpose to trace its phylogeny as well as its ontogeny we may proceed under the title of the Evolution of Clinical Pathology.

Examination of the urine dates back to antiquity. We read in Garrison's "History of Medicine" that the Babylonians performed this procedure under certain conditions as a religious rite where it was combined with inspection of the viscera of sacrificed animals as an essential part of the augury conducted by the priest-physician. Terra cotta models of the liver have been found whose age is estimated at 3000 years, which indicated that this organ was used as a basis for forecasting the future. The Babylonians believed that the liver was the source of blood, hence the seat of the soul which was offered to the god and to inspect the liver was to approach divinity receiving human favors. The inscriptions on these models suggest that they regarded disease as being caused by demons. This belief was shared, as we know, by practically every early race. Moreover, the Babylonians mixed their notions of medicine with astrology, the two being intimately interrelated. The Babylonians probably followed the teaching of the earlier Persians accord-

ing to Meunier who states that Zoroaster, a mythical priestly lawgiver, taught that the liver was the site of human emotion. On the other hand, if the Babylonians were not keen physicians they were good soldiers; they conquered the Hebrews, whom they placed in captivity about 604 B.C. The rigid dietary laws adopted by the Jews which necessitated the slaughtering of cattle according to prescribed rule, also the rigorous examination of the viscera of these animals is recorded in their sacred writing, the Talmud, where is found record of the postmortems they conducted on slaughtered



Die Urinprobe by Gerard Dous, reproduced with the permission of the publishers from Hollander, *Medizin in der klassischen Malerei*, 3. Auflage, 1923, Verlag von Ferdinand Enke in Stuttgart.

animals to determine their fitness for food. Diseased animals were regarded as "trepha" in contradistinction to certain portions of healthy animals fit for human consumption which were "kosher." But these inspections presuppose a fair knowledge of the normal and of some of the common diseases, and the writings in the Talmud indicate that the Jews possessed some knowledge of diseases of the lungs, liver, kidneys, intestine, mouth and other organs, although they shared many of the errors of the Babylonians and



their Egyptian contemporaries especially with reference to anatomy and physiology.

Egypt the land of hieroglyphics, has thus far revealed little advance in the knowledge of essential pathology. This is rather surprising since they practiced evisceration in the preparation of their mummies; further their papyri indicate a fair knowledge of many intestinal disorders especially the parasitic diseases. That some of our common diseases existed among the Egyptians has been definitely established by the recent identification of the pathologic lesions. Smith and Ruffer have described a case of tuberculosis of the spine in an Egyptian mummy whose age is estimated at 1000 B.C., but there is no evidence thus far that the Egyptians understood the nature of the disease. It remained for Hippocrates to associate this condition with pulmonary tuberculosis. Before him, however, Aesculapius, who tradition tells us was the son of Apollo, the Greek god of healing, had established temples managed by priest-physicians which approximated the modern health resort. Prayers and sacrifices were followed by baths, massage, catharsis, blood letting and other regimen; and when the grateful patient was cured he presented an offering to the temple which frequently was a model of the diseased part in wax or precious metal and so began the inception of the pathologic museum; although Bass states that this custom also existed among the Egyptians.

Hippocrates who lived 460-370 B.C. was not only the greatest of Greek physicians, but probably the greatest physician of all time. More than any other individual he helped establish the identity of medicine as a science and dissociated it from philosophy and theology of his age. He was the first to establish a definite system of pathology in which he attributed all disease to disorders of the several fluids of the body. He believed that there were four essential humors or body fluids,—blood, phlegm, yellow bile and black bile; that as long as they remained in proper proportions health was retained, while alteration in the normal ratio was manifested by disease. This theory, of course, collapsed as progress in medicine was made; but its spirit persists in the modern humoral theory of the mechanism of resistance to infectious diseases, and it is only befitting that one of the greatest of modern physicians, Paul Ehrlich, should be the exponent of this view. In addition, the writings of Hippocrates indicate a knowledge of tuberculosis, malaria, mumps, anthrax, puerperal septicemia and other conditions, although the observations are clinical rather than pathologic.

Galen lived in the second century of the Christian era. He combined the humoral theory of Hippocrates with the mathematical ideas of Pythagoras, reducing the whole structure of pathology to a series of exacting postulates in which the numeral four plays a most significant part. However, he gave us the four cardinal signs of inflammation—*rubor*, *tumor*, *calor*, *dolor*, according to Neuburger; also, he taught that there is no disturbance of function without organic lesion; further he was an uncompromising dogmatist, and prepared a huge encyclopedia of the medical knowledge of his day which for centuries afterwards was accepted as infallible. Galen

is generally cited as closing the period of antiquity which was followed by the Byzantine period which is conspicuous for its absence of progress in medicine. It appears as though the medical men of this age had been drugged by the galenicals of the previous era. Of interest is one Theophilus Protospatharius, a physician and a captain of the guard who wrote a treatise on the urine upholding Galen's view that the urine is derived from the blood being elaborated in the portal vein and vena cava.

The Byzantine period (476 to 732) is a cold-storage period in the history of medicine after which the leadership in medicine seems to have passed under Arabian and Jewish influence.

Rhazes who lived in the tenth century has given good descriptions of the contagious diseases, notably smallpox and measles, and Isaac ben Solomon, a contemporary of his, wrote a book on uroscopy which enjoyed a wide circulation for centuries. Another outstanding leader of this period is the Jewish physician, Avenzoar, who lived in the Western Caliphate in the twelfth century. He was a clear thinker and an independent observer. He wrote on pericarditis with effusion, mediastinal tumors, otitis media. Among other things, also he first described accurately the itch mite and Garrison calls him the "first parasitologist after Alexander of Tralles."

During the Middle Ages (1096-1438) pathology along with the rest of medicine slumped into a sort of quackery. Hand in hand with the wandering lithotomist, the barber-surgeon and conjurer of spells went the uromancer or the charlatan who proposed to arrive at all sorts of inaccessible material concerning the patient by the inspection of the urine such as the chastity of a woman, the sex of the unborn child, the condition of the liver, kidneys, heart and other organs. Undoubtedly many of these men were sincere in their prognostications, believing that they were based on reason and fact. On the other hand, the writings of others indicate their frank admission of quackery. According to Hollander, one Villanovanus, a leader of the school at Montpellier, taught his students in the year 1300 to make a diagnosis of obstruction of the liver when inspection of the urine failed to suggest any other condition. He further cautioned them that should the patient complain of a headache to insist that the headache came from obstructions of the liver; further, it was well to use such words as obstruction which the patients did not understand for to do so was to create a favorable impression. If the physicians of this period made little advance in medicine it was not through lack of opportunity. At no other period were there as many widespread epidemics claiming such a fearful toll of human life. The Black Death claimed millions; syphilis, thought to have been introduced by Columbus on his return trip, swept Europe like a pestilential epidemic, influenza, leprosy, epidemic chorea, ergotism and the sweating sickness each claimed its toll. The most enlightening phase of medieval medicine is the establishment of hospitals and the beginning of organized nursing.

The year 1453 marks the beginning of the Renaissance in medicine. This is the period of the revival of learning and the Reformation. A number of agencies were at work whose resultant force lifted the pall of the

medieval period. Among these may be mentioned the invention of the printing press, and the discovery of gun powder with its application in firearms, the discovery of America, and perhaps the bursting forth of long suppressed longing for individualism as opposed to feudalism. The outstanding medical personage of this period is Paracelsus (1493-1541) also called Aureolus Theophrastus Bombastus von Hohenheim. He has been called the most original thinker of the sixteenth century. Having studied alchemy, astrology and other occult sciences, he traveled about hobnobbing with barbers, executioners, midwives and fortune tellers. Nevertheless, in spite of his low tastes for company he was appointed professor of medicine at Basel in 1527. He discarded Galenism and the four humors,—he opposed uromancy and witchcraft,—he wrote on occupational diseases and correlated endemic goitre with cretinism and contributed a number of drugs which today are found in all pharmacopeias. Among his contemporaries were some famous anatomists who lifted this study literally out of the mire of public contempt and made it a living working science. Among these were Leonardo da Vinci, better known as a painter, Vesalius, the author of the epoch making "*De Fabrica Humani Corporis*" (1543), Sylvius, Fallopius, Eustachius, and others whose names are identified with our anatomical nomenclature.

One of the great leaders of this age was Ambroise Paré who contributed materially to the advance of anatomy, pathology, medicine and surgery. He popularized pathology in France by his numerous postmortem examinations including the bodies of persons of high rank among whom were no less than three kings, Henry II, who died following a lance thrust over the right eye which penetrated the brain, accidentally incurred at a court joust; the King of Navarre, who died from infection following a gunshot wound received during the Siege of Rouen; and Charles IX, who died peacefully from phthisis. Like Paracelsus, who discarded the classic languages and wrote in his native German, so Paré wrote in his native French. Born of humble parents he rose to the position of the first surgeon to the king. Near the end of his career he was the victim of a series of quarrels between the physicians, surgeons and barber surgeons of his day, necessitating his addressing a pamphlet to the Parliament of Paris in justification of himself and his work.

The seventeenth century is rich in the history of medical achievement. Harvey demonstrated the circulation of the blood and put physiology on a new basis. The microscope was coming into use, and enabled the investigators to study the finer structures of living matter which was destined to culminate in the enunciation of the cell theory two centuries later with the subsequent proclamation of the identity of protoplasm in all forms of life, both animal and plant.

One of the earliest microscopists was the Jesuit priest Kircher. This is of special historical significance in view of the muchly heaped abuse on the Roman Catholic Church, which has been accused of impeding medical progress. The sometimes quoted ecclesiastic bulls were in fact proclaimed



with the intent of protecting the bodies of dead soldiers from desecration. Kircher was probably the first to employ the microscope in the study of disease. He attempted to study the nature of the process of putrefaction by action of maggots in decaying matter; also, he made some observations on the blood of plague victims. A contemporary of his, Malpighi, has been called the greatest of microscopists and the founder of histology; he investigated the embryology of the chick and the histology of glands and viscera; he described the red blood corpuscles. His name is preserved in the Malpighian layer of the skin and the Malpighian corpuscles of the spleen and kidney. Redi opposed the view of spontaneous generation by showing that maggots did not appear in decaying meat when protected by wire gauze. Peyer described the hyperplastic intestinal glands of typhoid fever which bear his name; and chemistry was beginning to emerge from alchemy and the black art. Potable gold, the elixir of life, and the philosopher's stone had had their day and were being discarded. Physician and chemist were seeking an explanation of respiration which was to culminate in the discovery of oxygen the following century. Many important monographs appeared in clinical medicine and pathology, including observations on typhus fever, scrofula, scurvy, malaria, dysentery, rickets, ergotism, cretinism, apoplexy, glanders, pulmonary emphysema, asthma, angina pectoris, lead colic and numerous others. Of special interest is the first detection of albumin in the urine by Dekker of Leyden made by boiling urine in the presence of acetic acid, although glycosuria had been known almost from the inception of ancient medicine and had been described as "honey urine" by the early Hindus.

Uroscopy was falling into decadence but nevertheless was still a favorite theme of the artists who have preserved for us the visual impressions of the physicians of their day. Hollander describes a famous painting by Gerard Dous, a pupil of Rembrandt, in which a dignified old prosperous physician stands in a grey eape fringed with violet at an open gable window which is half covered by a Brussels carpet and through which light streams into the room from the side disappearing in a toned chiaroscuro. On the balustrade before him is a collection of articles connected with the profession of the healing craft including a parchment diploma with the seal of the university, a tin basin, a copper mortar, a globe, a folio volume, a viol, a candle, the beloved skull and the typical container of minor surgical instruments, while in the middle of the room is suspended a cupid as the guardian angel. The physician himself holds the glass flask in his hand inspecting the reddish urine which an old woman has brought. The eyes of both are directed to the test and in both there is an expression of sad resignation as the physician contemplates the specimen.

The eighteenth century is rather disappointing for its failure to keep pace set for its progress by the brilliant start of the previous century. There is something suggestive of feudalism attempting to assume its former influence, if not over the bodies of men, at least over their minds by systematizing all new thought into well regulated and organized classifications.



In spite of this inauspicious spirit of the time, clinical pathology made considerable progress. In fact aside from the Linnaeus' classification in botany, clinical pathology may claim practically all the significant observations of this century as occurring in its domain. In chemistry carbon dioxide was discovered by Black in 1757, hydrogen by Cavendish in 1766, nitrogen by Rutherford in 1772, chlorine by Scheele in 1774, oxygen by Priestley in 1771 and Lavoisier in 1775, and the composition of water by Cavendish in 1781.

In pathology, postmortem examinations were being introduced into clinics and a systematic attempt was being made to correlate the observed anatomical alteration with the clinical symptomatology; in serology observations were made on the coagulability of the blood by Hewson with reference to experimental delay and search was begun for the property of the blood causing coagulation, later to be identified as fibrinogen; and in bacteriology Jenner's work with preventive inoculation for smallpox was not only the great triumph of the age, but one of the greatest of all time. Beginning with the country-side yarn that milkmaids who contracted cowpox were immune from smallpox, Jenner attempted to transfer the immunity from a milkmaid to a boy by inoculating him with the matter expressed from a discharging lesion of the maid ill with the cowpox. When the boy was subsequently exposed to smallpox he was found to be entirely immune to it. Having collected 23 cases, Jenner published them in 1798 in a thin quarto under the title of "On Inquiry into the Cause and Effects of the Variolae Vaccinae."

The nineteenth century begins the modern period in medicine. Just as in the sixteenth century we find the great anatomists whose names have been incorporated into the anatomical nomenclature so we encounter numerous physicians in this era who described certain pathological conditions and whose names are now associated with them. Among them are Laennec, Graves, Stokes, Bright, Corrigan, Addison, Hodgkin, Parkinson, Jackson, Dupuytren, von Graefe and numerous others.

The cell theory was enunciated and established; pathology was placed on a cellular basis; chemistry witnessed the rapid discovery and isolation of numerous elements; the atomic and periodic tables were formulated; organic chemistry came into existence to be followed rapidly by physiological chemistry which paved the way for the newer blood chemistry; many of the infectious diseases were analyzed and the etiological factors demonstrated and the science of bacteriology was established; the numerous phenomena of immunity and serology were demonstrated and complement fixation was made a working principle; laboratory tests passed through a rapid succession of improvement, from the qualitative to the quantitative, and from the quantitative to the functional introducing the time concept into diagnostic methods and finally the more intricate laboratory tests as basal metabolism was introduced, the proper performance and interpretation of which requires a working knowledge of mathematics, physics, chemistry, pathology and clinical medicine. In fact so numerous and voluminous

were the significant discoveries that in a brief paper of this kind we can enumerate only a few of the more significant achievements.

Following the work of Kircher, Hooke, Malpighi, Grew, and others who made observations with the simple and imperfect microscope of the day, the early microscopists studying plant tissues saw small compartment-like spaces surrounded by a distinct wall filled with what they thought to be air or fluid and to which the name "cell" was applied. In 1831 Robert Brown directed attention to a small body found in the cell to which was given the name "nucleus" and in 1836 Valetin described the nucleolus within the nucleus. In 1838 Schleien proclaimed and demonstrated that plants were made wholly of cells and in 1839 Schwann declared that the animal body the same as plants was made up wholly of cells; in 1846 Mohl recognized the semifluid granular substance within the cell which he named protoplasm; in 1855 Virchow announced his famous aphorism "*omnis cellula e cellula*" and in 1861 Schultz proclaimed the identity of protoplasm in all forms of plant and animal life.

Virchow has been called the greatest of pathologists. In 1847 he founded the "*Archiv für pathologische Anatomie*" generally known as Virchow's Archiv; in 1856, he was appointed professor of pathology at the University of Berlin and in 1858 he published his "*Cellular-Pathologie*" in which he attacked the theory that there existed an invisible vital force distributed through the entire body or located within a few organs, instead this force was subdivided into an infinite number of individual associated vital forces which were located in the individual cells. "The cell," he wrote, "is actually the ultimate proper morphological element of every vital manifestation." He expressed the view that the body was a cell state in which every cell was a citizen and that disease was a conflict of the citizens within the state brought about by external forces, further he discredited the old view that there were distinct specific disease cells but regarded inflammatory and tumor cells as modifications of physiologic types. He wrote on a multiplicity of pathological conditions besides on archeology, anthropology, epidemiology and social conditions of his day. "The cellular theory," says Baas, "like all theories is to be regarded as a historical expression of the scientific tendency of a certain period."

In bacteriology perhaps the earliest observation was made by the Dutch microscopist, Anton van Leeuwenhoek, in 1683, who described the motility of organisms in scrapings from teeth; later O. F. Muller, in 1786, succeeded in discovering many structural details of microorganisms and gave descriptions of several kinds of bacteria, but it remained for Pasteur to establish the science of bacteriology. His researches in fermentation and spontaneous generation imparted to the study of bacteria a broad biologic importance. Pasteur demonstrated that putrefaction and decay which had been ascribed to spontaneous generation were caused by clinical disintegration of organic material due to the metabolic activities of bacteria whose primary interest was their search for food.

Koch was, perhaps, the first to show a specific relationship between a

bacterium and a disease. In 1876, he brought forth convincing proof that the bacillus anthracis was the cause of anthrax in cattle. In 1883, he discovered the cholera vibrio, and in the same year he described the organism of infectious conjunctivitis, now known as the Koch-Week's bacillus. He formulated "Koch's postulates" which prescribes the requirements for establishing the specificity of a casual microorganism to its disease.

He established definitely the etiology of tuberculosis although before him, in 1865, Villemin had shown that this disease was infectious. In 1871-4 Hansen established the causal relationship between leprosy and its organism; in 1879 Neisser called attention to the constant presence of a peculiar coccus in gonorrheal pus; in 1880 Pasteur observed staphylococci in pus from suppurative processes and in the following year Ogston described streptococci in inflammatory exudates; in 1883 Klebs described the diphtheria bacillus and in 1884 Loeffler emphasized its causal relation to the disease; in 1884 Frankel discovered the pneumococcus and in 1885 Leichtenstein saw the intracellular diplococcus in meningeal exudates; these were followed by numerous other significant discoveries including the organisms of glanders, erysipilas, tetanus, Malta fever, bubonic plague, puerperal infections and numerous others, and more recently, Hoffman and Schaudin in 1905 discovered the *Spirocheta pallida*.

In serology Brieger first isolated toxins in 1888, and in the following year Buchner demonstrated the bactericidal power of blood serum; later he showed that this property existed in the serum. In 1890, Behring and Kitasato demonstrated that rabbits and mice could be artificially immunized against tetanus and that this immunity was associated with the ability of the blood plasma to render harmless the toxic substance produced by the tetanus bacillus which substance they called "antitoxin," previously, in 1888, Nuttall showed that blood loses its bactericidal power when heated to 56° for one-half hour; in 1894, Pfeiffer demonstrated bacteriolysis in vivo, and in the following year Metchnikoff and Bordet demonstrated that this lysis occurred in the test tube if the organisms were mixed with the serum of an animal previously injected with the organisms.

In 1896, Pfaundler drew attention to the peculiar phenomenon of agglutination which occurred when bacteria were grown on immune serum and in the same year Widal and Grunbaum turned these facts to practical use in the diagnosis of typhoid fever. In 1897 Kraus described the bacterial precipitins—he observed that when the serums of animals that had been immunized against specific organisms were added to a clear filtrate of the respective bouillon cultures of their bacteria, the clear solution became turbid and a precipitate formed. Nuttall in a very painstaking research showed that the blood of practically every animal could be identified by its specific precipitin reaction, also, he showed the blood relationship of various animals based upon group precipitins.

In 1898, Bordet demonstrated that red blood cells would undergo lysis both in animals and in the test tube provided the animals in which the red cells were injected or from which the serum was drawn, had previously



been inoculated with homologous red cells; further he demonstrated that three constituents were necessary—the specific red cell, the serum of the animal injected with these and a constituent of all normal serum discovered by Buchner and called alexin by him and now called complement. Neisser and Wechsberg, in 1901, showed that complement not only acts quantitatively in being fixed but that it can be deviated. Upon these principles the complement-fixation test developed with bacterial suspensions used as the first antigens, later to be replaced by various fractional modified extracts.

In chemistry, the nineteenth century witnessed enormous progress. It is reasonably safe to state that in this century more progress was made in this science than in all previous time, and the twentieth bids fair to eclipse the nineteenth. It had been taught for many centuries that matter was made up of small indivisible particles, but it remained for Dalton to propound a truly chemical atomic theory, declaring that the atoms of the elements are not of the same weight and that the relative atomic weights of the elements are the proportions by weight in which the elements combine. In 1803, he published his first table of atomic weights bearing out this view. The atomic values given by Dalton have since been shown to be incorrect, but he paved the way for others. In 1818 Berzelius announced his theory of chemical proportions and made use of chemical symbols and formulae as they are employed today. Previously in 1809 Davy described the nature of alkalies and Gay-Lussac described the law regulating the combination of gases by volume, and Avogadro, in 1811, declared the law that bears his name that under constant pressure and temperature, the number of molecules of a gas in a given space is always the same. In organic chemistry, Wöhler, in 1828, prepared synthetically urea from inorganic constituents and bridged the gap between the inorganic and organic substance, and later, he showed that benzoic acid taken by mouth appears in the urine as hippuric acid. This experiment is sometimes cited as the beginning of the chemistry of metabolism. A few years earlier Prout, in 1824, demonstrated that the essential acid of the gastric juice was hydrochloric acid, Liebig, a pupil of Gay-Lussac, helped to establish the science of organic chemistry by his studies of cyanides, cyanates, hippuric acid, chloral, chloroform, amides, aldehydes and numerous others. Synthetic organic chemistry made enormous strides during this century and one of the greatest contributors to this branch was Fischer.

The study of chemical metabolism of the body was developed and threw great light on the diverse functions of the body in health and disease, especially the constitutional disorders; yet there were many problems that were unexplained. The science of nutrition was greatly facilitated by the discovery of the isodynamic law which states that a given amount of food if taken into the body may liberate practically the same amount of energy that it would produce if burned in oxygen outside the body; this permitted the application of the measurement of food by a method similar to the method used in measuring the energy derived from coal. An important contribution was made in 1906 by Hopkins who observed that growth of the body required something in addition to the established requirements of carbohydrates, pro-



teins, fats, salts, water and oxygen. He called them hypothetical substances "accessory food factors" and in 1911 Funk coined the word "vitamines." The study of vitamins has thrown light on such conditions as scurvy, beriberi, rickets, and other conditions.

The development of colorimetry enabled the quantitative estimations of substances and amounts heretofore regarded as impractical of measurement by the chemical balance and volumetric methods, and hand in hand with this new system of chemical analysis went the development of the newer methods of blood chemistry as the quantitative determination of blood, sugar, nonprotein, nitrogen, creatinin, uric acid, urea and other substances. It is significant to us that most of the observations in this branch were made by American observers. Prominent are the names of Folin, Benedict, Van Slyke, Meyer and others. The information which these blood chemical methods have rendered available is especially important since the older method of examination of these conditions by cytology, bacteriology and serology gave insufficient information. They have been especially helpful in the analysis of the constitutional diseases as diabetes mellitus, nephritis, gout, renal diabetes, etc.

Clinical laboratory methods went through a rapid succession of improvements. Emerging from the old art of uroscopy which made use of inspection primarily, chemical reactions came into use and established definitely the presence or absence of certain substances.

In 1848, Fehling published his quantitative test for sugar in the urine. This test and its modifications have acquired a time honored place in the laboratory because of the ease of manipulation and definiteness of results and it is probable it will continue to remain one of the commonly employed methods. Qualitative data were improved when quantitative methods were introduced because knowledge of the amount of sugar gives greater information of the disease than merely information as to the presence of sugar. A quantitative test of the amount of sugar in the blood is still more valuable because it approaches closer to the site of the pathological lesion and a functional sugar tolerance test such as recommended by Haman and Hirschman represents the highest type of biochemical test so far introduced because it measures the working capacity of the body to utilize the sugar in question.

The significant feature of the functional test is the introduction of the time concept into laboratory methods. The functional test is always made with reference to the time of something else such as the time of taking of food, the time of administration of drugs, the time of performing exercise, etc. In this respect the functional test is in conformity with Einstein's theory of relativity which links up the time element as a sort of fourth dimension to our previous three dimensional concept; and with the introduction of the time element certain standards previously considered absolute now become relative; for instance, 0.14 per cent blood sugar may be normal or it may be pathological depending upon its relationship to the time of taking of food. If the test is made within two hours of food taking, it probably is normal, but if it occurs twelve hours after food taking it is probably pathological.

The same relativity holds true in the other biochemical functional test. The single gastric analysis is no longer regarded as satisfactory. According to the observations of Hawk, Rehfus and Bergheim, it is unsatisfactory to interpret the data obtained from the examination of the single test meal removed at one hour interval as this represents but one phase in a constantly changing cycle of gastric digestion and it is impossible to judge what has preceded or what has followed. A given figure may be a part of normal, increased or decreased gastric curve.

In this paper there has been no attempt to trace the evolution of pathology itself but only the evolution of laboratory methods. Disease existed in earliest primitive man and before him in the vertebrate mammals, and before them in the earliest organisms that lived countless millions of years ago. Bacteria existed in early pre-Cambrian era and the fossil remains of nitrifying coccoid organisms have been found in the mountains of Montana by Wolcott, the age of these mountains being estimated at 32,000,000 years, but the exact knowledge of bacteria may be said to have begun with Pasteur a few decades ago.

Finally it appears that history must repeat itself. Just as we have read of the wrangles between the barbers, the barber-surgeons, the surgeons and the physicians of a past age each trying to maintain its caste, so we encounter the comments of a few self-appointed censors today. In the December 2, 1922, issue of the official journal of our good mentor the American Medical Association the statement is made by some nameless individual who hides his identity under the cloak of the Advisory Advertising Committee of the Board of Trustees of this Association and writing anonymously states that "the status of the clinical pathologist is not the same as that of the internist or surgeon. The latter deals with variables—human beings—the former conducts manipulations on fixtures—inanimate substances." This writer evidently was thinking of the famous painting by Dous showing the physician of a past age inspecting the urine. One is inclined to wonder whether he ever saw a basal metabolism test performed, or a functional sugar determination requiring the removal of a sample of blood every half hour for three hours, or a fractional gastric analysis or any other of a multiplicity of tests requiring very exacting control of the patient.

The clinical pathologist differs from the internist it is true but not according to the standard mentioned by the nameless one. The clinical pathologist does not treat disease, he confines himself to the performance of the more exacting diagnostic measures.

If any lesson is to be learned from the history of medicine, it is that one of the first duties of the physician is to understand the nature of disease, and in this respect the clinical pathologist functions as a true physician.

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## THE EFFICIENCY OF RADIATION THERAPY\*

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BY U. V. PORTMANN, M.D., CLEVELAND, O.

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SINCE the advocacy and development of deep x-ray therapy by German specialists, roentgenologists in the United States have eagerly accepted their teachings and with characteristic enthusiasm have begun the treatment of all types of malignant conditions. Some of our best qualified roentgenologists have investigated the methods employed in European Clinics and have indorsed them with qualifications, which to a certain extent have been overlooked by many who are now attempting to employ deep x-ray therapy. It was because of our inexperience in the field of deep x-ray therapy that it became necessary to accept the work of European physicists and other investigators as the primary basis for the work in America. But unfortunately the methods employed in Europe are not entirely adaptable to our own different working conditions. In particular our American apparatus for the measurement of intensity and our generating apparatus are not the same as theirs. The American manufacturers of x-ray apparatus have made an attempt to meet the demand by marketing high voltage machines, with a resultant unfortunate competition and commercialism of a valuable but dangerous weapon.

We found that European methods were not always applicable to conditions here, but European physicists are now revising and retracting some of their earlier work so that now their results more nearly conform with later results of experiments performed by Americans.

In our own Clinic the experimental application of physical principles to

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the work of the x-ray department is conducted by a physicist, Hugo Fricke, Ph.D., who very early called our attention to certain discrepancies between our results and those first reported from Europe. Dr. Fricke uses an electroscope of his own design made in the mechanical department of the Clinic. This is well protected from possible sources of error. The ionization chamber is of organic substance and its material and design were chosen after considerable study and experimentation. The investigation was conducted under our own working conditions with standard American apparatus. A room adjoining the therapy department made it possible to conduct the experimental work under conditions identical with those under which the therapy would be applied. All factors were checked by approved methods of measurement.

Very many factors enter into the correct interpretation of measurements made by the ionization method. Dr. Fricke carefully studied the published reports and found in them certain errors which were corrected in our own laboratory so that now we feel that our work is as exact as is possible. For example, as our work progressed we learned that our equal intensity curves though almost similar in configuration gave much less of a depth dose (10 cm.) than those published. In fact, we were unable to obtain a depth dose approaching 34 per cent under any practical working conditions. Since determining our own intensity curves we have found that other workers have come to a similar conclusion regarding the earlier reports: that is, that the depth of x-rays under practical working conditions is considerably smaller than was originally determined. The results of Dr. Fricke's work are to be published at an early date.

When applying our equal intensity absorption curves in the treatment of patients we found it impossible by any method of application to administer a deep "carcinoma" which I call a therapeutic dose, or say a 120 per cent dose, to an average case such as an uterine carcinoma. We found that in such a case not less than five portals of entry as large as 10 by 15 cm. each are necessary to deliver a deep dose of from 85 to 100 per cent. In increasing the size of portal to more than  $20 \times 20$  cm. very little difference in depth dose results and the larger size is impracticable. Even if we use factors giving the maximum or 34 per cent dose, on account of dosage received at opposite or neighboring portals the total dose delivered at one or more portals must be diminished, thus losing a percentage equal to the amount of that diminution at the required depth.

If the conclusions of the physicists are correct and the many reports of apparent cures are well-founded, we must conclude that deeply situated pathologic lesions yield to a smaller dose of radiation than has previously been supposed. But it is highly improbable that deeply situated carcinoma yields to a smaller dose than does superficial carcinoma and we know that the cure of most malignant growths requires a very large dose of radiation. If radiation therapy has any place in the treatment of malignancy and we know it has, it must be given in large doses at one time. It is possible, therefore, that some of the apparent cures reported from x-ray therapy alone, have



really had insufficient radiation and later disappointment in the results of deep x-ray therapy will develop.

For ourselves, as the result of our short, practical experience and from the findings in our experimental investigation, we believe that with our present methods it is impossible to give a sufficient therapeutic application of x-ray radiation to most deeply situated malignancies in one dose. But we have learned also the inadvisability and danger of insufficient radiation and of repeated doses of x-ray.

It is absolutely essential that each case be thoroughly studied not only as a biologic but as a physical problem, i.e., the physical laws of radiation must be properly applied to each individual case. The average roentgenologist is poorly equipped to make the essential studies of the physical laws which are involved; he has not the time, the apparatus, or the special knowledge, each of which is necessary. We believe that the empirical application of x-ray radiation to patients with malignant diseases is detrimental—some apparently good results notwithstanding. There is no question as to the value of radiation therapy—the only question is as to the proper method of application.

Fortunately in any case in which the radiation which can be applied to a malignant tumor is therapeutically insufficient the valuable assistance of radium can be employed to supply the percentage of the dose that is lacking. Dr. Henry Schmitz and others have frequently called attention to the combined method of radiation. Even with the quantities of radium ordinarily available it is possible to administer a therapeutic dose of radiation if the radium and x-ray intensity curves are understood and properly applied. By superimposing radium intensity curves over x-ray intensity curves through the necessary portals of entry, we are able to build up an evenly distributed and homogeneous therapeutic dose of radiation not only to the tumor itself but also to distant areas that may or may not be malignant. Thus we approach the technic of the surgeon who has learned the necessity not only of removing malignant tumors, but of making also a wide-spread dissection. It is probable that those who rely upon either x-rays or radium alone, will be very much disappointed in the ultimate results. Moreover we are not yet ready to state that there is no specific cure for certain malignant diseases in x-ray or radium. Our best weapon is the judicious combination of surgery, radium and the x-rays in the proper proportions for each individual case. It should be borne in mind that while surgery seems to have reached the limits of its application to the treatment of malignant diseases, the use of radiation is still in its infancy. The value of radiation has been demonstrated, but in the present stage of our knowledge we cannot foresee what may be its final potency. Until that limit has been demonstrated we must be prepared to use every available weapon in our attack upon malignant tumors.

The problem of radiation therapy is to determine and administer a maximum therapeutic dose of x-rays in combination with a proper dose of radium, and in making this determination it must be constantly borne in mind that while the x-ray radiation is diffuse, the sphere of radium radiation is within this area; therefore, care must be taken that in the resultant overlapping of

the maximum intensities of each the surrounding normal structures are not permanently damaged. Radium may be applied by whatever methods are preferred by the radiologist, but the equal intensity curve for whatever method is employed should be understood. In our Clinic the radium is applied by Dr. T. E. Jones who prefers to use rather large quantities of radium and arranges the needles with great care so as to obtain a widely homogeneous radiation of distant pathologic areas. The x-ray radiation is administered in an intensity sufficient to build up an even therapeutic dosage at least 10 cm. from the point at which the radium is inserted.

Unfortunate results of overradiation by deep x-rays have been reported, but these must be due to too frequently repeated treatment. The danger of insufficient radiation is almost as great. In our experience those cases which were not properly irradiated at the first treatment did not do well following subsequent treatments and the end results have not been as good as when a properly measured dosage was applied at the first radiation. Damage to the adrenals has been reported. Whether or not radiation produces fibrosis of the lung is unsettled. Some workers state that fibrosis of the lung has not occurred in their experience; but we have observed several cases in which it did follow full doses with only one-half millimeter of copper filter. On enquiry several roentgenologists state that they have lost cases from afebrile "pneumonia" about six weeks after radiation which is the period of time after which we have observed the fibrosis to develop. It may be that the lighting up of a latent infection is responsible for this condition. On the other hand we have subjected tumors of the lung to radiation without subsequent ill results.

#### SUMMARY AND CONCLUSION

From our study and experience we conclude that:

- (1) In order to obtain the best results in the treatment of malignant diseases by radiation the physical principles of the production and action of the x-rays and of radium must be understood.

- (2) It is usually impossible to deliver a therapeutic dose of x-ray radiation to deeply situated areas. The large deep doses which were first reported are not obtainable under present practical working conditions.

- (3) Radiation by either the x-rays or radium alone is insufficient: they should be used in proper combination.

- (4) The danger of insufficient radiation is as great or greater than that of overradiation; the desideratum is the administration of the proper dose at the first treatment.

## BACTERIOPHAGE PHENOMENA. I\*

LLOYD ARNOLD, M.D., CHICAGO, ILL.

DURING the past five years there have been a great many publications dealing with the "bacteriophage phenomena." We do not wish to go into the literature on this subject, as this has been already reviewed by several authors in this country, Davison,<sup>10</sup> Kuttner,<sup>4</sup> d'Herelle-Smith.<sup>5</sup> We wish only briefly to mention the methods that several workers have used in obtaining the "bacteriophage substance."

D'Herelle<sup>1</sup> inoculated bouillon with the fecal material from a patient convalescing from dysentery (*B. dysenteriae shiga*). After 18 hours incubation this was passed through a Chamberland filter. Several drops of this filtrate was added to a young bouillon culture of *B. Shiga*, causing a complete clearing of the tube after a short period of incubation. A suspension of *B. shiga*, obtained from a young agar culture, added to this cleared tube, and incubated overnight, again became clear. One drop of this cleared or lysed culture was sufficient to cause complete clearing of a young culture of *B. shiga*. This could be transmitted in series.

Bordet and Ciuca<sup>2</sup> showed that this same "bacteriophage substance" could be obtained from other sources than the stool filtrate. These workers injected a strain of *B. coli* into a guinea pig intraperitoneally, three or four times at intervals of a few days. The exudate so obtained was diluted with broth and allowed to stand at room temperature for a day or so and then filtered. This filtrate when added to a normal culture of the *B. coli* produced lysis transmissible in series.

Kuttner<sup>3, 4</sup> obtained a "bacteriophage substance" from glycerol extracts of the intestine, and the acetone precipitate of the liver of guinea pigs, also from normal rabbit serum.

Some time ago we found that the washed suspension of the surface of an agar culture of irregular or bacteriophage colonies when passed through the Berkefeld filter, contained a strong bacteriolytic substance.

We found that if the whole of the agar, after the surface lycogenic growth had been washed off, was transferred to a flask and shaken with either distilled water, normal salt solution, or bouillon, and then passed through a Berkefeld candle, this filtrate showed a stronger bacteriolytic activity than the surface washings. Both of these filtrates cause lysis transmissible in series and did not differ in any way from the bouillon filtrate prepared by the d'Herelle method in so far as the "bacteriophage phenomena" was concerned.

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There was a large amount of protein material in the bouillon in addition to the biproducts of metabolism of the young culture of bacteria and when cleared by the d'Herelle method, the bacterial protein content must be considerably increased. The washings of the surface growth of the lycogenic culture contained bacterial proteins. The agar, remaining after this washing, when extracted with water contained some bacterial proteins and also the bouillon base used in the agar preparation. We found that we could repeatedly extract this agar and each time remove in the extracted aqueous medium some bacteriolytic substance. The first extract was bouillon colored, the second a trace of this coloring, the third was usually water-clear, the fourth and remaining extracts were always clear. We have extracted the same agar as many as twelve times, and even in the twelfth extracts found a "bacteriophage substance."

For some of the experiments to be reported in this paper, and for a future publication dealing with some questions of immunology, we have used the following method for the preparation of our "bacteriophage" or bacteriolytic principle.

Washed and dried agar was added to distilled water to make a 2 per cent concentration. This was heated until dissolved and filtered, then autoclaved in convenient sized flasks. Ten c.c. of this water-agar was poured into a sterile petri dish. After cooling a piece of thin sterile tissue paper, 0.5 mm. less in diameter than the inside measurement of the dish, was laid on top of this sterile water-agar layer. Then 7 or 8 c.c. of standard nutrient agar was poured on top of this tissue paper.

The surface of this nutrient agar was seeded with a lycogenic strain of bacteria and incubated for twenty-four hours. By means of sterile forceps the tissue paper with the nutrient agar layer can be removed as one piece without contaminating the under layer of water-agar. This lower layer is then removed, pressed through an ordinary coffee sieve to break it up into small particles. Ten c.c. of water is added for each agar plate used, also a little toluol, and put in the shaking machine for 8 to 12 hours, then in the ice box overnight. The fluid is strained off through glass wool and a coffee sieve, filtered through wet filter paper to remove the toluol, and then passed through the Berkefeld filter. The agar is returned to the flask, and equal amount of water is added, shaken, etc., repeated.

The filtrates obtained from these bottom layer water-agar extracts contained a bacteriophage substance, transmissible in series, inactivated at 75° C. and conformed in every way with the original d'Herelle bacteriophage. The fourth extract is water clear, the first and second contain bouillon absorbed from the upper layer. The third extract is sometimes bouillon tinged in color.

The extracts are active up to the twelfth one, after this there was only a trace of bacteriophage present and after the fifteenth extract the bacteriophage activity was negative.

D'Herelle found that the precipitate formed upon the addition of 9 parts of alcohol contains a substance causing lysis, but this was not trans-



missible in series, and he regarded this to be the "lysin," the active principle extracted from the "bacteriophage." The precipitate formed upon the addition of 9 parts of alcohol to either of the first three extracts does not contain any substance causing lysis. Upon the addition of alcohol to the fourth and remaining filtrates there is no precipitate formed, although they contain a very active bacteriolytic agent.

We have run controls in the following manner: The bottom layer of water-agar was extracted after incubation without inoculation, and also extracted after the upper nutrient agar layer had been inoculated with *B. typhosus* (Olsen). In neither instance was there a bacteriolytic substance obtainable.

The original source of the bacteriophage used by us was isolated from the stool of a case of typhoid fever by d'Herelle's method. This was actively bacteriolytic against several strains of *B. typhosus* and also against 8 of our 12 strains of *B. dysenteriae*. This will be referred to as B.S. in this page.

Feces of a normal two and one-half year old baby yielded a good bacteriophage against several strains of *B. dysenteriae* (Shiga, Flexner, Hiss, Sonne B, Strong). This will be referred to as B.B. in the future. (This bacteriophage and also the Olsen strain of *B. typhosus* was kindly furnished us by Dr. Kuttner.) The Olsen bacteriophage was used by us before we had the opportunity of isolating any of this substance for ourselves. This was designated as our B.Z. strain of bacteriophage.

#### EXPERIMENTS

Six tubes of bouillon, each containing 10 c.c., were inoculated with a loop of an 18-hour old bouillon culture of *B. typhosus* (Olsen). One-half c.c. of the B.Z. bacteriophage was added to each of 5 of these tubes. After thorough shaking, a loop of the first tube was seeded on the surface of a sterile agar plate and smeared well with a bent glass spreader; then the remainder of the contents of this tube was immediately passed through a Berkefeld filter. The other 4 tubes were placed in the incubator. At intervals of three hours, plates were inoculated and the contents of the respective tubes were immediately filtered as was done with the first tube. The remaining tube was plated out every three hours as a control for the relative amount of growth. The plates were incubated 24 hours. The ratio of the regular to the irregular colonies was recorded.

The plate from the first tube showed the ratio of the regular:irregular colonies as 80:20, second plate 5:95, third plate 80:20, fourth and fifth plates were regular colonies throughout. The filtrates from the respective tubes were pipetted into freshly inoculated bouillon cultures of several members of the typhoid-dysentery group to the concentration of 1:10, 4 strains of *B. typhosus*, 3 strains of *B. dysenteriae* (Flexner), 2 strains of *B. dysenteriae* (Shiga), 1 strain each of Strong, Hiss Y and Sonne B. were used. Filtrates from tubes 1 and 3 showed inhibition in only one typhoid strain (Olsen), one Flexner, and in Hiss and Sonne B. for 18 hours, after this growth appeared and remained cloudy for the period of observation (21 days). The filtrate 2, that is the 3 hour growth tube, inhibited all the strains used for 18 hours.

Of the typhoid strains, the Rawlings' and Hopkins' strains showed growth after 36 hours, the Olsen and Mt. Sinai after 96 hours. Two Flexner, one Shiga, the Strong, Hiss and Sonne B. strains were inhibited during the period of observation. The remaining strains showed growth either after 36 or 48 hours. The filtrates from tubes 4 and 5 showed an inhibition of the Olsen typhoid strain for 18 hours. All the remaining tubes were as cloudy as the controls.

This experiment has been repeated dozens of times, using hourly plating and filtrates instead of every three hours. We have used the B.S. and B.B. bacteriophages, the surface washings of plates showing irregular colonies, the agar extracts of these plates, and also the bottom-layer-agar extract. The results usually agree with these mentioned above. Some few variations occur and these will be treated more in detail later.

The B.B. bacteriophage, isolated originally from a healthy baby's stool, was usually active against *B. dysenteriae* (Flexner, Hiss, Strong and Shiga), in the order named. This strain of bacteriophage was not active against any of the strains of *B. typhosus* ordinarily, but the 3 hour bouillon filtrate of this bacteriophage with a susceptible dysentery strain was active against two of the four *B. typhosus* strains. d'Herelle,<sup>5</sup> Gratia,<sup>6</sup> Bail,<sup>7</sup> Doerr<sup>8</sup> and others have reported an increased production of the bacteriophage, upon incubation with a susceptible strain of bacteria, after the second or third hour. They mentioned an increased virulency of the bacteriophage for the susceptible strain of bacteria used. We have found this to be true and accompanying this is a bacteriolytic action against strains of the whole typhoid-dysentery group. We have not found any of these bacteriophages, even at their maximum virulency, to be active against gram-positive bacilli or cocci.

We have found that there is a parallel between the relation of regular to irregular colonies on an agar plate seeded from a bouillon culture containing a susceptible strain of bacteria with a bacteriophage and the virulency of the bacteriolytic substance in the tube at the time of seeding. As the irregular colonies increased in number the virulency or activity of the bacteriophage, at the time the agar plate was inoculated, was found to be increased. Twelve tubes of bouillon were seeded with *B. typhosus* (Olsen). To one tube a bacteriophage was added in the concentration of 1:10 and an agar plate was inoculated from this culture, before and immediately after the bacteriophage was added. After one hour's incubation, bacteriophage was added to another tube and plates inoculated before and after the addition, as was done in the first instance. This was repeated each hour for ten hours. In this way we added bacteriophage to cultures of a susceptible microorganism at the time of inoculation and at each hour up to and including the tenth hour of growth. These plates were incubated 24 hours and the character of growth recorded. Plates were again inoculated from these same tubes after 24 hours, 48 hours, 14 and 25 days. The results obtained from this experiment were more variable than those of the former one mentioned. We wish to give the average result obtained from 46 such experiments using a bottom-agar-layers of the B.S. bacteriophage.

The first plate, that was inoculated from the tube of bouillon seeded

with *B. typhosus* and the bacteriophage added immediately, did not contain many irregular or lyceogenic colonies, seldom over 10 to 20 per cent. The second plate, seeded from a tube after one hour's incubation and the bacteriophage added, showed more irregular colonies, usually 25 to 35 per cent. The third plate showed that about 50 per cent of the colonies were irregular; the fourth plate, about 80 to 90 per cent were irregular colonies; the fifth plate, that is after 4 hours' growth and the bacteriophage added at this time, was usually sterile. The next three plates were usually either sterile or showed a high percentage of irregular colonies. The remaining plates usually showed good, heavy growths; the irregularity observed in these plates corresponds to the "appearances" described by American and English workers; "tâches vièges" by the French and "Loecher" by the Germans, in this field. The ratio between these clear areas, or "appearances," and the regular heavy growth was somewhat variable, the regular colonies usually predominated.

Inasmuch as we have observed the gradual increase in the number of

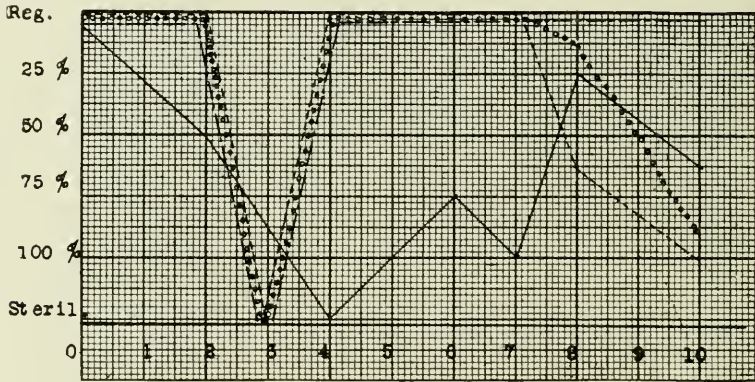


Fig. 1.—Abscissa is the age of the *B. typhosus* (Olsen) in hours at the time the bacteriophage was added.

Ordinate is the percentage of irregular colonies on the agar plate as compared with the regular normal growth.

Immediately after addition of ——— bacteriophage. - - - After 24 hours' incubation of the tubes. o o o After 48 hours' incubation of the tubes. — . — . After 14 and 25 days' incubation.

irregular colonies, as compared with the regular colonies, on the plates inoculated from young bouillon cultures of *B. typhosus* when a bacteriophage is added, we were led to believe that the susceptibility of the bacteria is greater at the hours of incubation which produce the most irregular colonies, or even sterile plates. The filtrate from tubes showing the maximum amount of irregularity on plates were much more virulent than the filtrates from the tubes showing more regular growth.

This has led us to record our results in graphic form. The abscissa is the time in hours of the age of the bouillon culture of the bacteria at the time the bacteriophage was added. The ordinate, from above downwards, represents the degree of irregularity of all the colonies present expressed in percentages, from regular colonies (i.e., no trace of bacteriolytic activity), 25 per cent, 50 per cent, 75 per cent, 100 per cent and sterile. We do not wish to convey the idea that a culture will pass through all of these stages



gradually in an orderly manner. Sudden sterility has been observed, also a sudden return from a sterile plate to one showing a good growth of regular normal colonies. We have been using this chart method in recording our results in this laboratory for the past two years, and we wish only to use this method of expression in order to save space and show clearly our results. (Fig. 1.)

It will be observed that after 24 hours' growth the 3 hour old culture of *B. typhosus* (Olsen) was rendered sterile by the addition of the bacteriophage.

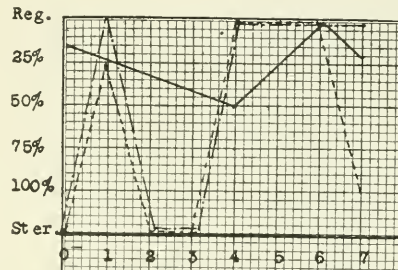


Fig. 2.

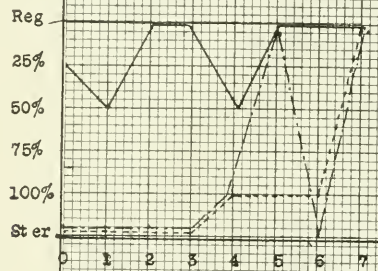


Fig. 3.

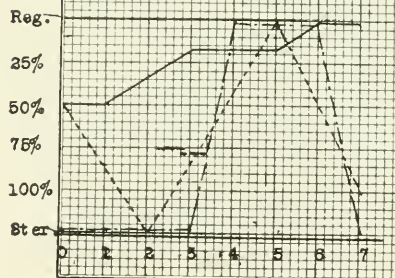


Fig. 4.

The 8 and 10 hour old cultures after 24 and 48 hours' incubation show decreasing amount of bacteriophage activity. The 14 and 25 day old cultures show that an equilibrium has been reached, only the 3 hour old culture tube shows any change and this remains sterile.

The descent of the curves from all of the ages of the cultures after the seventh or eighth hour, up to the time equilibrium is reached, from a regular colony growth to varying degrees of irregularity and even sterility, is striking. There is usually a heavy growth, especially in the immediate plating, with clear areas or "appearances." We think that d'Herelle and others,



who have thought these "appearances" to be "bacteriophage colonies" and have by an enumeration of such appearance attempted to determine quantitatively the bacteriophage present, have been using this very variable factor as a constant.

The same technic was followed in this experiment as was described in the preceding one. Four sets of tubes were used, one set was inoculated with an 18 hour bouillon culture; one from a suspension in bouillon of colonies from an agar culture 24 hours old, one from a 48 hour old agar colony suspension, and the remaining set from a 72 hour old agar colony suspension in bouillon. The bacterial suspensions were made in bouillon to compare in density with those of the 18 hour bouillon culture; Gate's method in determining density of the suspensions was used.<sup>9</sup> (Figs. 2, 3 and 4.)

We give the results of this experiment to show that the age and also the medium upon which the susceptible microorganism has been grown, all must be taken into consideration in dealing with a "bacteriophage substance." This is particularly true when one uses such methods as d'Herelle and others have used for determining quantitatively the amount of bacteriophage present. Without a knowledge of the source of the culture and its history preceding the time it is put in contact with the bacteriophage, any quantitative estimation based upon the number of "appearances" or clear areas on the agar plate are valueless. We do not think that enough is known about this phenomena to enable one to attempt quantitatively to determine the amount of "bacteriophage substance" present in any given medium.

#### SUMMARY

"Bacteriophage Substance" or the bacteriolytic agent is adsorbed into the agar upon which the lyogenic colonies are grown.

A method is given to obtain a filtrate containing an active bacteriolytic substance that is free from proteins of the bouillon or nutrient culture medium. The addition of alcohol does not cause any precipitate in these filtrates.

A bacteriolytic substance added to a 3 hour old culture of susceptible bacilli of the typhoid-dysentery group causes a marked increase in the lytic power of this substance, not only for the homologous strain, but also for all other members of this group. The bacteriolytic activity is not only influenced by the age of the bouillon culture to which it is added, but is also dependent upon the age of the culture used to inoculate the bouillon medium and as to whether it was grown on a solid or fluid medium before inoculation.

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# THE EFFECT OF A NORMAL MEAL UPON THE BLOOD SUGAR LEVEL IN HEALTH AND IN CERTAIN CONDITIONS OF DISEASE\*

## A SIMPLE FOOD TOLERANCE TEST

BY I. C. BRILL, A.B., M.D., PORTLAND, OREGON

THIS study was undertaken with a view to developing a simple method for the determination of abnormal glyceimic reactions. It is intended as a substitute for the glucose tolerance test now in general use in clinical and metabolic laboratories. This latter test is the result of the work of many authors, notably Bang, Hopkins, Hamman and Hirschman, and Janney and Isaakson. The value

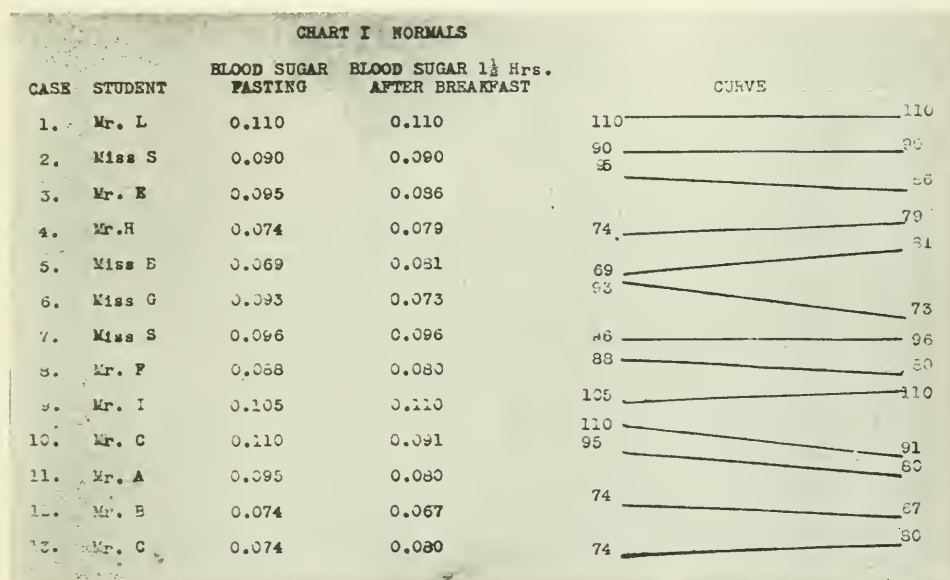


Chart I.—The results of the glyceimic reactions in thirteen normal individuals—all healthy students of the University of Oregon Medical School. It will be noted that the lines obtained are almost horizontal. The highest rise is 12 mg., while some present even a slight fall.

of the procedure as an aid in the study of various metabolic disorders is too well recognized to require further comment at this time. Some three years ago, while studying a number of cases of suspected and of some early known diabetes, it occurred to me that the use of a comparatively large quantity of glucose (which is necessary in the performance of the glucose tolerance test) is not entirely free from objections. First, the mixture is nauseous and distasteful to the patient; second, there may be some element of danger (theo-

\*From the University of Oregon Medical School, Portland, Oregon.  
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CHART II MILD DIABETICS

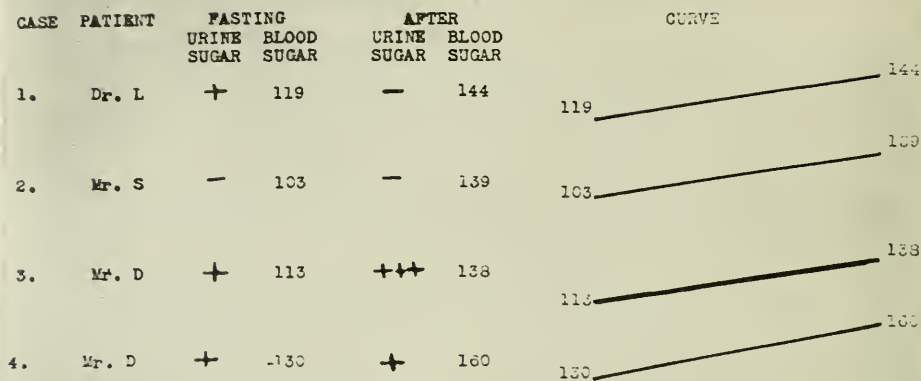


Chart II.—The result in known mild diabetes. Note all show a definite rise of from 25 to 36 mg. The fasting level is also apt to be higher than in the normal, although in mild cases it may not be so. In this group the fasting level was within normal limits in three of the four cases.

CHART III DIABETES BEFORE TREATMENT

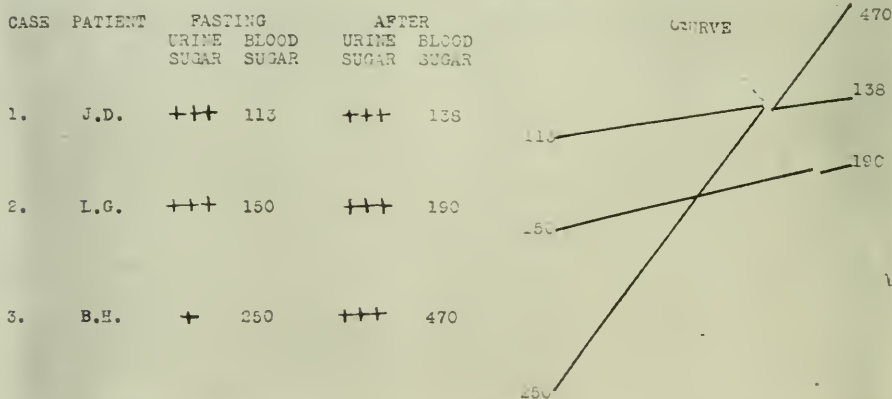


Chart III.—Results in three cases of diabetes, mild, moderately severe and severe, respectively.

CHART IV SAME CASES AS CHART III AFTER TREATMENT

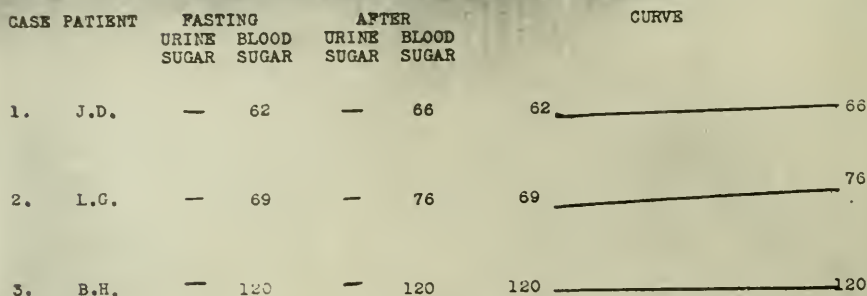


Chart IV.—Results in same three cases as shown in Chart III after treatment.

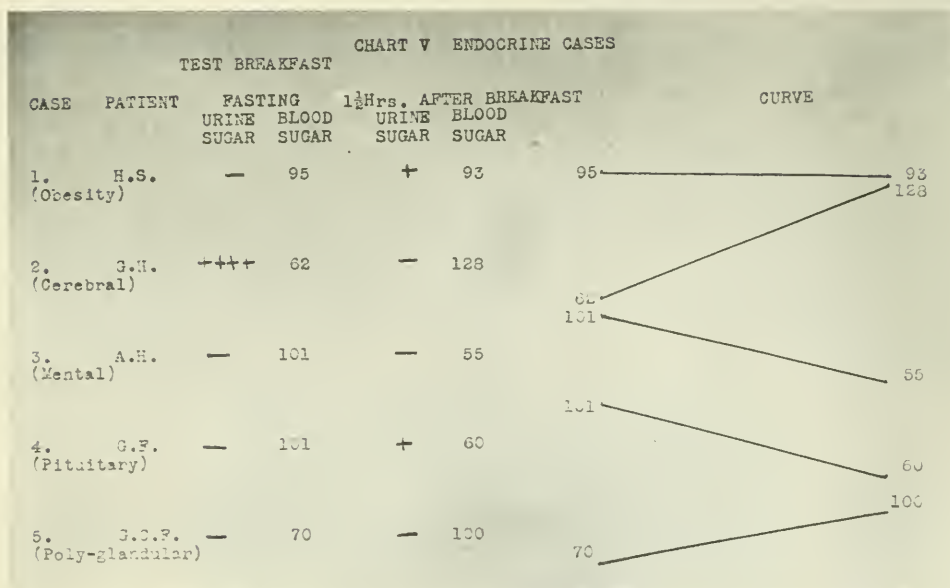


Chart V.—Results of the study of five endocrine cases. One of these cases showed nothing abnormal except for obesity. It will be noted that her glycemic reaction was practically normal. The other four cases all showed definite endocrine disorders and they all gave characteristic irregular glycemic reactions.

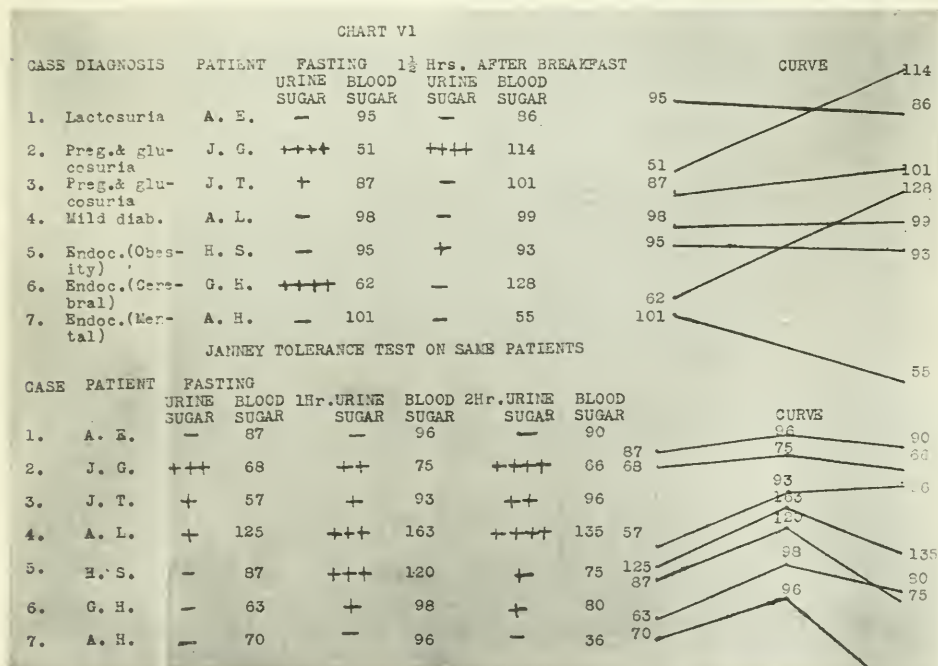


Chart VI.—The results of seven cases in which this test was used as an aid in establishing the diagnosis. In all these cases the findings were further checked up with the glucose tolerance test and as may be seen from the chart, the results were quite parallel. Two of these cases deserve special notice. They were cases of pregnancy with well marked glucosuria. (Cases 2 and 3). The question of terminating the pregnancy came up in each instance. Upon the basis of the findings by our test, I recommended to allow the pregnancy to continue. Only moderate restriction in carbohydrates was advised. Both cases were carried to a successful delivery without the slightest mishap. Both the mother and the child in each instance are living and well today.



retically, at least) in overloading a weakened tolerance by rapid and sudden absorption of a comparatively large quantity of pure glucose. These considerations led me to make a comparative study of the effects of an average normal breakfast upon the blood sugar content in normal individuals and in various chronic conditions in which the food tolerance is known to be affected. The results obtained are presented in the accompanying charts. The studies here recorded were all done in the latter part of 1919 and early part of 1920. Since these records were compiled, numerous other cases were similarly studied with identical results. However, in order to avoid repetition of figures and for the sake of clearness, only the original charts are here presented.

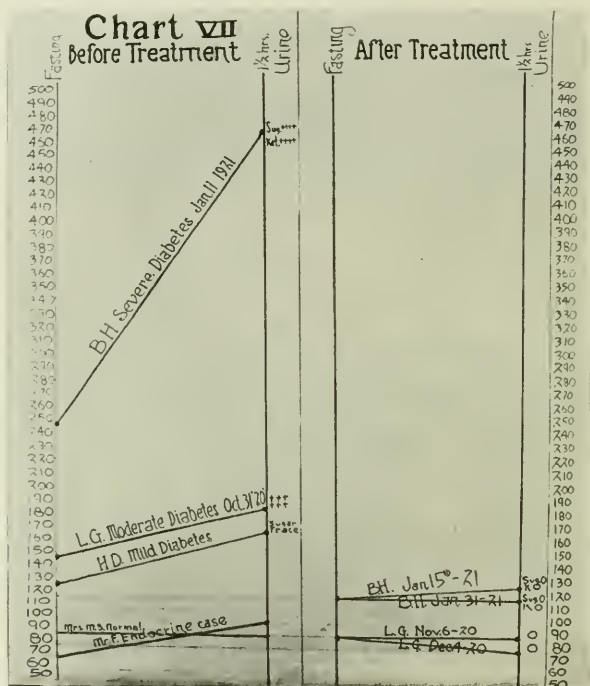


Chart VII.—Summary of the previous charts. On the left side of the mid-line curves are shown of the cases before they were treated. The dates of the estimations and the diagnoses are indicated. On the right side of the mid-line, the same cases with the dates of the subsequent estimations are shown. The same scale is used in order to show the marked contrast in the curve in different conditions, and in the same conditions before and after treatment.

In making this comparative study, the following factors were considered:

First, *the time*. It was shown by Mosenthal and others that in diabetes the maximal percentage of blood sugar occurs between one and two hours after breakfast and that after luncheon and evening meals the rise is not so constant and the sugar may, in fact, even fall. On the other hand, in normal individuals, I have found the blood sugar to be most nearly constant after the morning meal, while, after luncheon, it is apt to rise higher than normal. Breakfast time, therefore, will afford the greatest contrast between the normal and the abnormal conditions.

Second, *the meal*. In order that the test may be a true food tolerance test all the food elements should be represented in the test meal, and it therefore included the usual breakfast foods, namely: bread, butter, cereals, eggs, sugar, coffee and cream, amounting to approximately 100 grams of carbohydrates, 26 grams of protein, 27 grams of fats and 760 calories.

Third, *the curve*. Two estimations only were done, fasting and an hour and a half after breakfast. These two estimations form a line which may be horizontal, rising or falling. Normally an hour and a half after breakfast the blood sugar is within ten milligrams of the fasting level and the two estimations furnish almost a horizontal line. In diabetes, however, a greater difference is found, the second level being considerably higher than the first and, therefore, a rising line is obtained. The difference may best be learned by an examination of Charts 1 and 2.

#### CONCLUSION

1. A comparatively simple method is suggested to serve as a true tolerance test.

2. In all the cases tried so far, it corresponds very closely in its results with the Janney glucose tolerance test.

3. It is preferable to the glucose tolerance test because (a) it is more simple, (b) it is more agreeable to the patient, (c) and it avoids the danger of overtaxing a weakened function by a large quantity of pure glucose.

I wish to express my thanks to Prof. Howard D. Haskins from the department of Biochemistry of the University of Oregon Medical School for assisting me in many of the blood sugar estimations, the results of which form the basis of this study.

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#### THE DIFFERENTIAL LEUCOCYTE COUNT IN CHRONIC PERIAPICAL DENTAL INFECTION.\*

BY RUSSELL L. HADEN, M.D., KANSAS CITY, MO.

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AT the present time the radiograph is necessarily the main dependence in the diagnosis of chronic periapical dental infection. Clinicians and dentists agree that patients suffering from systemic disease of local origin should have removed all teeth which may be a source of infection. There is a great difference of opinion, however, as to what should be done with pulpless teeth which are negative in the radiograph, and even with teeth which show evidence of infection in the absence of systemic disease. The problem is of great concern to both the physician and dentist. It is of special interest to the dentist since the patient presenting himself to him is so often not suffering from systemic disease, while the reverse is true of a patient coming to the physician.

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From the Deane Institute.

The criteria of systemic disease are relatively crude and the physical signs relatively gross. Great damage may be done to body tissues before the changes are serious enough to be recognized by the usual clinical methods. It is apparent that foci which are causing general damage should be removed early as a preventive measure. Some diagnostic sign which would enable us to determine whether absorption is taking place from infected areas in the absence of systemic disease would be of the greatest value in indicating the proper procedure to follow with pulpless teeth. Such teeth may be negative in the radiograph and yet be infected, since there must be many bacteria present before sufficient bone destruction takes place to show in the x-ray. On the other hand teeth around which there is evident bone destruction may have become sterile and thus be harmless from a systemic standpoint.

Attempts have been made to determine toxin absorption from infected foci by the complement-fixation method. Mixed cultures or organisms isolated from periapical infection are used as an antigen. Such procedures have yielded little information of value. A study of leucocytes of the blood would seem to be a more promising method of approach, since the white corpuscles may respond quite rapidly when bacteria or products of bacterial activity are absorbed by the blood.

Hecker states that he has observed tinctorial changes in the leucocytes in the presence of dental infection.<sup>1</sup> Logan found a leucocytosis in 47 out of 52 cases of periapical infection.<sup>2</sup> Goadby thinks that there is constantly an increase in mononuclears in pyorrhea and systemic disease.<sup>3</sup> Daland concludes that "small cell lymphocytosis with a corresponding decrease in the polymorphonuclear cells is an important diagnostic sign of periapical dental infection, the value of which is increased when leucopenia exists."<sup>4</sup> This observer considers that lymphocytosis (38 per cent or more of lymphocytes) indicates that toxins or streptococci or both are entering the blood stream. He states that he found a lymphocytosis only twice in 100 cases of chronic disease where no focal infection existed. Toren claims to have found a certain type of leucocyte in the blood indicative of infection about the teeth.<sup>5</sup> This cell, he thinks, originates in the lymph glands draining the infected area.

To determine whether we could utilize the findings described by others as a diagnostic measure in chronic periapical dental infection, I have made a differential leucocyte count on blood films from 200 patients. The films have in every instance been made on cover glasses, a procedure which allows the drop of blood to spread spontaneously and evenly. It is very doubtful whether it is possible to get a true differential blood count where films are made on slides. No films have been used unless there was a uniform spread of the cells with the red cells one cell thick over an area covering at least 8 low power fields of the microscope. On each film 500 cells have been counted, hence the study includes a count of 100,000 cells. All the counts have been done by the author.

The films have been made on  $\frac{7}{8}$ " cover slips and stained with Wright's stain as follows: eight drops of stain are run on the film and left for one minute, eight drops of a phosphate buffer solution of  $P_H$  6.4 are then added and left

for four minutes. The film is washed with distilled water, dried and mounted film side down in balsam. We have found that the addition of the buffer solution instead of distilled water to the stain gives remarkably uniform preparations and differentiates very sharply the various types of white cells. The granules of the granular cells are brought out especially brilliantly in films prepared in this manner.

In such a study it is apparent that the basis of classification of the leucocytes should be plainly stated. This is not clear in many published reports. Daland, for instance,<sup>4</sup> appears to have divided the cells only into lymphocytes and polymorphonuclears. Toren in claiming that there is a peculiar type of cell in infection about the mouth evidently uses a different classification from other hematologists, since he finds in normal individuals only 1-2 per cent large mononuclears while others agree on 6-10 per cent.

From the standpoint of origin there are three different types of white cells in the blood (1) polymorphonuclears coming from the bone marrow, (2) lymphocytes, large and small, arising in the lymph glands and (3) true monocytes, which include both the large mononuclears and transitionals. The origin of the latter is in dispute, but the consensus of opinion is that they are of endotheloid origin. The identification of the polymorphonuclears is simple. The same may be said of the small lymphocytes. There is a great deal of confusion, however, concerning the relation and differentiation of large lymphocytes and large mononuclear cells. In films stained by the technic described above, the mononuclear cells are sharply differentiated. The large lymphocytes have dark blue nuclei and the protoplasm is clear or diffusely blue. The protoplasm may contain azurophile granules, which are a very dark purple color, are usually few in number and show a great variation in size. The true large mononuclears, on the other hand, have relatively larger nuclei which may be round, indented, or saddle-bag shaped and usually take a lighter stain than the nucleus of the lymphocytes. The protoplasm has a ground glass appearance due to the uniformly distributed light purplish blue granules which are very fine and of uniform size. The granular appearance has been ascribed as due to nodosities in the reticulum of the protoplasm, but seems more likely due to the presence of true granules.

The classification we have used has the added value that it can be compared with the very complete study of the normal differential leucocyte counts made by Miller.<sup>6</sup> Miller includes the large lymphocytes with the large mononuclears and makes a separate classification of the transitionals. The lymphocyte group is thus smaller and the large mononuclear group larger than would be when the cells are classified on the basis of origin.

Patients have been grouped as follows:

Group I—Includes 134 patients who showed evidence of alveolar abscess in the dental radiograph. Since we have proved by culture that many such abscesses are sterile it does not necessarily follow that all the individuals of this group really have periapical infection.

Group II—Includes 66 patients who showed no definite evidence of infection in a radiograph of the teeth. 28 of the 66 patients in this group had



one or more pulpless teeth. Many pulpless teeth which show no radiographic evidence of infection are really infected, so here again the grouping is rather artificial.

Group III—Comprised 47 patients who showed, on careful physical examination, definite evidence of systemic disease of focal origin. The diseases in-

TABLE I

GROUP			I	II	III	IV	V
TOTAL NUMBER OF COUNTS			134	66	47	53	25
Average			8311	7800	8783	7714	8838
Total White Blood Cell Count	Distribution of Counts	Over 10,000	11.8	7.5	27.6	9.5	24.0
		8000-10,000	42.2	33.3	36.2	30.3	44.0
		6000-8000	38.5	52.8	29.8	45.5	20.0
		4000-6000	7.5	6.1	6.4	15.2	12.0
		Average	64.4%	62.2%	63.9%	64.6%	66.2%
Polymorpho- nuclear Neutrophiles	Distribution of Counts	Over 80%	2.2	1.5	2.5	0.	0.
		70-80%	23.9	4.2	12.7	40.0	16.0
		60-70%	47.9	34.8	63.8	32.1	60.0
		50-60%	23.9	36.4	19.2	22.7	24.0
		40-50%	2.2	3.0	2.0	5.2	0.0
Average			1.7%	2.0%	2.3%	1.6%	2.5%
Polymorpho- nuclear Eosinophiles	Distribution of Counts	Over 5%	3.0	3.0	6.3	0.	8.0
		4-5%	3.0	3.0	6.3	0.	8.0
		3-4%	8.2	6.1	15.0	9.4	8.0
		2-3%	20.2	30.3	27.7	24.5	36.0
		1-2%	44.8	31.8	25.5	41.5	16.0
		Under 1%	20.8	25.7	19.2	24.5	24.0
		Average	0.5%	0.4%	0.4%	0.4%	0.5%
Polymorpho- nuclear Basophiles	Distribution of Counts	Over 1%	3.8	6.0	10.6	16.9	16.0
		0.4-1%	73.1	72.7	59.5	37.7	28.0
		Under 0.4%	23.1	21.3	29.9	45.5	56.0
		Average	27.1%	28.5%	26.0%	27.5%	26.6%
Lymphocytes (large and small)	Distribution of Counts	Over 30%	29.9	45.5	23.4	35.9	28.0
		25-30%	31.3	24.2	25.5	17.0	40.0
		20-25%	21.6	18.2	38.3	24.5	16.0
		10-20%	16.4	12.1	12.8	22.6	16.0
		Under 10%	0.8	0.	0.	0.	0.
		Average	6.2%	6.5%	6.5%	5.9%	6.3%
Large Mononuclears and Transitionals (Monocytes)	Distribution of Counts	Over 10%	4.5	6.0	10.6	7.5	8.0
		8-10%	5.9	12.1	23.4	9.4	24.0
		6-8%	14.2	21.2	23.4	15.1	16.0
		4-6%	67.2	53.0	19.2	54.7	24.0
		Under 4%	8.2	7.6	23.4	13.3	28.0

cluded are arthritis, neuritis, iritis, pyelonephritis, duodenal ulcer, encephalitis and endocarditis. All patients had pulpless teeth and 36 had periapical abscesses.

Group IV—Includes 53 patients who were thoroughly examined and found to have no evidence of systemic disease; 29 of this group had pulpless teeth and 17 had periapical abscesses.

Group V—Comprises 23 patients, all of which were proved by culture to have infected teeth. In every case rabbits were injected and the lesion from which the patient suffered reproduced in the animal. There is every reason to believe that the dental infection was the source of the systemic disease. If the differential blood count shows anything typical in focal infection it should certainly be evident in this group.

The results of the differential counts are shown in Table I. The average white cell count in Group I is 8311 per c.mm., Group II, 7800; Group III, 8763; Group IV, 7714, and Group V, 8838. The differential count is remarkably alike in the five groups. The average polymorphonuclear neutrophile is 62.2 per cent in Group II and 66.2 per cent in Group V. These are the two groups which one would expect to show the greatest difference. The eosinophiles show much the same variation, being highest in Group V. The basophiles show no change. The lymphocytes show practically the same average throughout the five groups. The average is 26.6 per cent in those patients showing undoubted evidence of disease of focal origin (Group V) and 28.5 per cent in those showing no evidence of infection in the radiograph (Group II). The large mononuclear cells show equally little variation.

#### DISCUSSION

The results in this series of counts indicate that the patient with alveolar abscesses will have a slightly higher total white count than one who shows no radiographic evidence of infection. If systemic disease of focal origin is present the difference is somewhat more marked. This difference is not great enough, however, to be of any definite value in diagnosis and decision as to treatment.

A study of the differential counts shows that the increase in white cells observed is due to a general increase in all types of cells, although there is a slight relative increase in the polymorphonuclear cells. There is no relative increase in the lymphocytes and large mononuclear cells. We have found no evidence of any type of cell which is peculiar to focal infection.

There is no sufficiently clear-cut increase in any one type of cell to make the differential leucocyte count of definite value in the diagnosis of absorption from areas of infection. In fact, the study has demonstrated to us that the differential leucocyte count is of little value in our problem.

#### SUMMARY

The results of the study of the white cell count and differential count in 200 patients is presented; 100,000 white cells have been counted.

Patients with periapical abscesses show a slightly higher total white count than those having no abscesses. The difference is somewhat more marked in those patients suffering from systemic disease of focal origin.

The increase is, for the most part, in the polymorphonuclears, although all types show some increase.

Patients suffering from chronic periapical dental infection do not show typically a lymphocytosis.

We have found no evidence of an unusual type of white cell or tinctorial reaction in chronic dental infection.

The differential count is of little practical value in determining whether a patient is absorbing toxins or bacteria from possible foci of infection about pulpless teeth.

Group I. Patients showing in radiograph definite evidence of one or more infected teeth.

Group II. Patients showing in radiograph no evidence of infected teeth.

Group III. Patients with infected teeth and systemic disease of focal origin.

Group IV. Patients with no evidence of systemic disease of focal origin (17 showed definite areas of rarefaction in dental radiograph, 28 had no pulpless teeth, and 18 had pulpless teeth showing no evidence of infection).

Group V. Patients with systemic disease of focal origin which was reproduced in rabbits by bacteria isolated from periapical foci.

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## CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

BY A. LEVINSON, M.D., CHICAGO, ILL.

(Continued from page 678.)

### STOOL

THE first requirement in the interpretation of stool of infants is to examine it immediately after or only a short time after it has been defected from the infant. Changes in color and consistency take place in stools on standing. Often a stool that is light yellow or deep yellow in color to start with, will take on a greenish or real green color on standing one or more hours. A stool that has been watery to start with often dries up on standing, leaving no residue or leaving only traces of stain on the diaper. One must also take into consideration the type of food ingested by the infant, especially the sugar, as the food influences the color, size, and consistency.

The next condition in examination of infant's stool is that no cathartic or laxative should have been given the baby on that day, for every cathartic changes the appearance of the stool. Castor oil, for instance, produces mucus in the stool even in a normal baby, and calomel gives a green color, whether or not there is intestinal disturbance to begin with. What is true of physics is also true of other medications. Charcoal, for instance, shows black particles in the stool and argyrol colors the stool brown.

**EXAMINATION.**—The most important points in the examination of infant's stools are: number, size, consistency, color, odor, curds, reaction, fat, mucus, pus, blood, bacteria, and parasites. Size, color, and curds are determined by the naked eye. Reaction is determined by litmus paper, or by titration (easiest by litmus paper); fat is determined by sudan III or galactometer. Blood is determined macroscopically, microscopically, or by the guaiac test; pus is determined microscopically. Bacteria are determined both on slide and on culture media, special tubes and special media being used for culturing stools (see section on smears and cultures). Parasites are determined by the naked eye or by the microscope, parasitic ova are determined microscopically.

The number of stools in healthy infants and children varies between 1 to 3. Some infants and children will, however, have only one bowel movement in 36 hours with untoward effects on their health. The food ingested also influences the number of stools. Boiled milk tends to constipate, vegetables tend to increase the number of stools.

The size of the stools depends on the age of the child; the number of stools, the character of the food, and the condition of the gastrointestinal tract. Infants' stools are necessarily smaller, as infants' food contains relatively less residue. When the diet is increased by cereals and vegetables, the stools increase in size because of the increased residue. In cases of starvation and partial intestinal obstruction, such as pyloric stenosis, chronic intussusception, and malformation of the rectum, the stools are small. In dilatation of the colon, Hirschsprung's disease, the stools are infrequent and very large—constipated stools tend to be large. In most cases of diarrhea the stools are small and numerous. In tenesmus due to any cause the stools are frequent and small.

The consistency of the stools varies with the diet and condition of the digestive tract—the thin fluid stool seen in diarrhea, often on relatively high carbohydrate feeding; the normal semifformed or soft stool seen in thriving infants and children; the firm formed and even hard stool in constipated children.

Color depends a great deal on the type of food ingested and on the time the stool has been standing before examination. Breast feeding gives a deep yellow stool. Cow's milk alone, or with cane sugar, also gives a yellow color. The addition of dextri-maltose to the milk changes the stool to a light brown, Mellin's Food changes it to a dark brown, and honey changes it to a light yellow.

One way of testing whether the stool has been green to begin with, or has become so on standing, is to examine the inner part of the stool. If the green color is due to oxidation, only the outside will be green, the rest being yellow. If the green is formed in the intestine, this color will be seen all through the stool. Green stool (the color being present in fresh stool) occurs in nearly all cases of diarrhea.

Normal infant's stool has no odor at all or it smells "sour." An offensive odor indicates disturbed digestion.

Large bean-shaped curds are often present in the stool when an infant



is fed on raw milk. As soon as the milk is boiled or sodium citrate is added to the milk, the large curds disappear. Small curds varying in size from a pinhead to a linseed are present even in normal stools. When present in large numbers they may be indicative of disturbed fat digestion.

The reaction of a normal stool is neutral, faintly acid, or faintly alkaline. This may be ascertained by the use of litmus paper. If the stool is formed a suspension may be made with a small portion of stool and a few c.c. of distilled water. Diarrheal stools tend to be acid because of the presence of fermentation products. A high carbohydrate intake favors fermentation. When large amounts of fat are ingested, the resulting fatty acids tend toward acid stools. When high protein feedings are given there is putrefaction with resulting alkaline stools.

Fat in stools may be seen as neutral fat, fat soaps, and fatty acids. To examine for fat, a small amount of stool is smeared on a glass slide and a drop of sudan III stain added. This colors neutral fat bright red. Soaps do not stain. Fatty acids stain orange red. When a weak solution of carbol-fuchsin is used instead of sudan III the soaps stain a faint rose, the neutral fats do not stain, and the fatty acids are red. The crystals of fatty acids may be seen by adding a drop of acetic acid to the smear, and heating for a minute or so, and then allowing it to cool.

The presence of starch may be demonstrated by the addition of iodine which gives the starch granules the usual blue-black color.

Mucus may be seen as white, shiny, tenacious masses of varying amounts. Pus may be observed both macroscopically and microscopically.

Blood in the stool may impart a dark or tar color to the stool, as is the case when the hemorrhage is high up in the intestinal tract. Fresh blood, from rectal fissure, polyps or ulcerations low in the intestine, appears bright red and is not thoroughly mixed with the stool. Sometimes blood is present in very small amounts and can be determined only microscopically or by chemical tests.

**THE GUAIC TEST FOR BLOOD.**—A water suspension of stool is prepared and one-third volume of glacial acetic acid is added and thoroughly mixed. If blood is present the coloring matter is thus converted to acid hematin. The mixture is now filtered and extracted with two or three volumes of ether. Fresh tincture of guaiac is prepared by dissolving a knife point of powdered guaiac in 5 c.c. of alcohol. About 2 c.c. of the ether extract is treated with 10 drops of the tincture of guaiac and 25 drops of hydrogen peroxide are added. After thorough mixing the presence of blood is evidenced by a blue color which fades after standing.

**PARASITES.**—Worms, especially round worms and tape worms, can be detected with the naked eye. Pin worms can also be found if looked for carefully. The mother may best do this when the child goes to bed. In looking for tape worm segments, the search can be facilitated by tying a large strip of gauze over the opening of the bed pan in which the stool is to be obtained. This is placed in the sink under running cold water for an hour or two and the parasites are looked for in the residue. In looking for ova, a substantial

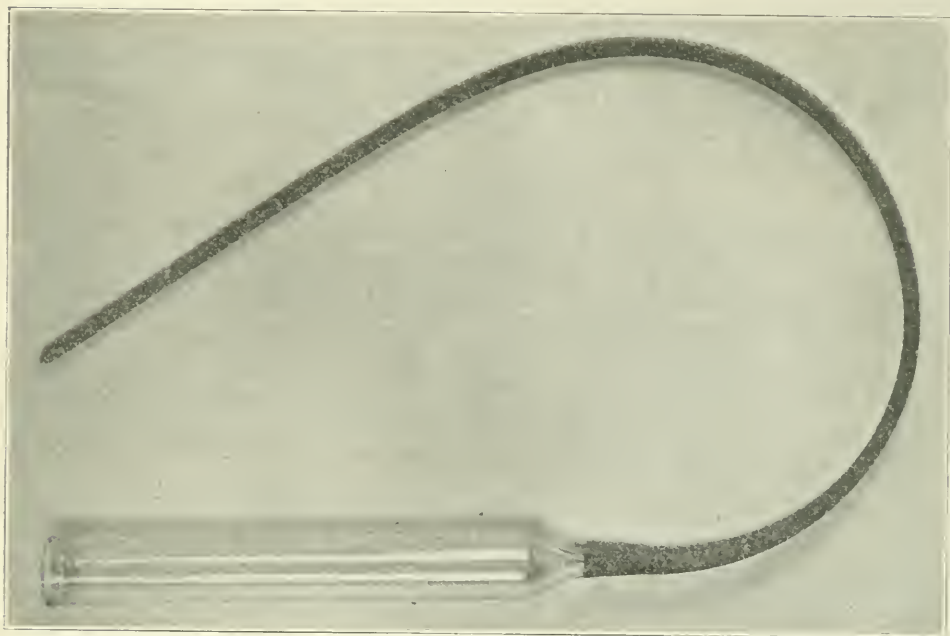


Fig. 30.—Barrel of glass syringe and catheter used advantageously for gavage.



Fig. 31.—Gavage.

amount of the stool is rubbed up thoroughly with several volumes of a saturated chloride solution. The parasites rise to the surface, are scraped off with a spoon, and set aside for one hour. The surface of the fluid is now skimmed with a wire loop  $\frac{1}{2}$  to  $\frac{2}{3}$  inches in diameter, and several loops placed on the slide. This is examined under low power.

### LAVAGE AND GAVAGE

*Indications.*—Lavage is indicated in cases of severe vomiting due to food disturbances, especially when the vomitus contains large quantities of mucus. It is also indicated in poisoning. It is employed occasionally in older infants and children who refuse food because of neurosis.

Gavage is indicated when the child refuses food; when he is too weak to take food; when there is a deformity of the mouth, such as in marked cleft palate; in cases of stricture of the esophagus; in profuse persistent vomiting; and in postdiphtheric paralysis.

*TECHNIC.*—The child is wrapped in a sheet and held firmly on the nurse's lap, or laid down flat on the table. The tongue is depressed with a tongue depressor, if the child has teeth, or with the index finger of the left hand if the child has no teeth. The catheter held between the thumb and index finger of the right hand is now passed quickly into the esophagus; the index finger of the left hand in the pharynx being used as a guide for the catheter.

The catheter should be soft, 12 to 14 French for infants, and 18 to 20 French for older children, especially if gavage is to be given, and especially if thick food, such as cereal, is to be introduced into the stomach. An idea of the length of the catheter to be inserted may be obtained by measuring the distance between the tip of the child's nose and the ensiform cartilage.

A funnel is attached to the catheter by means of an extra piece of rubber tubing, and a short glass tube used as a window. The glass part of a medicine dropper may be conveniently used for the latter.

For lavage, a 2 per cent sodium bicarbonate solution, 1 per cent to 2 per cent boric acid solution, or plain warm water is used. The solution is poured into the funnel and allowed to run back by lowering the funnel. The procedure is repeated several times until no more food or mucus comes back with the solution.

For gavage the food is introduced into the funnel and allowed to run into the stomach gradually. The barrel of a ten c.c. glass syringe attached directly to a catheter will answer the purpose for gavage. (Figs. 30 and 31.)

A rubber apron for the physician and nurse, and a large basin as a receptacle for the stomach contents in lavage will facilitate the procedure.

Nasal feeding may have to be resorted to in cases of paralysis of the pharynx, especially in acute anterior poliomyelitis and postdiphtheric paralysis.

The technic of nasal feeding is the same as in the ordinary gavage, except that the catheter is introduced through the esophagus by way of the nostril, instead of by way of the pharynx.

## ESTIMATION OF THE AMOUNT OF PANCREATIC ENZYMES IN DUODENAL FLUID BY A MODIFIED GAUTIER'S METHOD 1\*

BY GEORGE F. SPENCER, M.D., PHILADELPHIA, PA.

MUCH has recently been written concerning the estimation of the gastric secretions with a determination of the acidity or the alkalinity. The duodenum has been explored by means of the duodenal tube and samples of duodenal secretions have been procured and examined both chemically and microscopically<sup>1</sup> but apparently the duodenal secretions, with particular reference to the pancreatic enzymes, have been practically overlooked.

One or two methods for estimating enzymatic activity in duodenal contents have been published<sup>2</sup> but apparently no practical method for the busy clinician has been brought forth. For that reason we propose to give you a method<sup>3</sup> for the determination of the pancreatic enzymes in duodenal fluid which is simple enough for any clinician to use, accurate enough for practical purposes and one which requires the minimum of time and expense to perform.

*Procedure:* Modification of Gautier's method.

(A) Prepare three 50 c.c. Erlenmeyer flasks marked T, A and L (trypsin, amylase and lipase).

(B) To flask T. add 20 c.c. of a 5 per cent solution of gelatine (1).

(C) To flask A. add 20 c.c. of a 5 per cent solution of soluble starch (2).

(D) To flask L. add 5 c.c. 20 per cent emulsion of olive oil (3).

(E) To each flask add one or two drops of a 1 per cent alcoholic phenolphthalein solution. Then measure into each flask by means of an Ostwald pipette 1 c.c. of the duodenal fluid to be tested.

(F) To each flask add drop by drop, from a burette, decinormal sodium hydroxide until a light pink color is produced which remains on shaking. At times it will be necessary to add, in the same manner, decinormal hydrochloric acid instead of sodium hydroxide, for, at times, the reaction will be found to be already alkaline, that is alkaline to phenolphthalein. Incubate for one hour at 37° C., shaking the flasks every fifteen minutes.

(G) Upon removal from the incubator, at the end of exactly one hour, place flasks T. and L. in ice water and add to flask A. a few grains of sodium carbonate to stop digestion.

(H) Controls of boiled duodenal fluid, plus gelatine starch and oil treated as above stated must be incubated with the three tests and correction for their blanks be made.

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Corring—Borden—Keen Research Fellow of the Jefferson Medical College.



## ESTIMATION OF THE TRYPTIC ACTIVITY. CALCULATION

*Direct acidity.* Titrate gelatine digestion mixture to neutrality, (first light pink) with 0.1 N. NaOH, take the burette reading, subtract the blank reading: The result is a measure of the direct acidity developed by the ferment activity of 1 c.c. of the duodenal fluid upon one gram of gelatine during one hour's digestion at 37° C.

*Total Acidity.* Add 5 c.c. of neutral formol alcohol solution (4), titrate again to neutrality. Take the burette reading, subtract the blank reading. The result plus the figure for the direct acidity, is a measure for the total acidity due to the amino acids derived from the action of one c.c. of the duodenal fluid upon one gram of gelatine.

## ESTIMATION OF THE LYPOLITIC ACTIVITY. CALCULATION

*Direct Acidity.* Titrate oil emulsion digestion mixture to neutrality with 0.1 N. NaOH, take the burette reading, subtract the blank reading. The figure thus obtained is a measure of the direct acidity developed by the action of one c.c. of duodenal fluid acting upon one c.c. of olive oil.

*Total Acidity.* Add 10 c.c. of neutral alcohol-ether solution (5) titrate again to neutrality, take the burette reading, subtract the blank reading. The result is a measure of the acidity developed by the fatty acids derived from the action of 1 c.c. of duodenal fluid upon 1 c.c. of olive oil for one hour at 37° C.

## ESTIMATION OF THE AMYLOLYTIC ACTIVITY. CALCULATION

(1) *Maltose.* Pour starch solution digestion mixture into a 25 c.c. burette, run slowly, then drop by drop, into 25 c.c. of Benedict's reagent to which has been added 10 to 15 gm. of sodium carbonate. Estimate the amount of maltose present by Benedict's Quantitative Method for dextrose (6). Take the burette reading and note the number of c.c. required to cause a complete disappearance of the blue color of Benedict's Reagent (5). This figure divided into 0.0747, (the amount in grams of maltose required to reduce 25 c.c. of Benedict's Reagent) gives the amount of maltose in 1 c.c. of digested starch solution. Multiply by 20 to obtain the amount of maltose formed from one gram of soluble starch (5 per cent solution used).

(2) *Weight of starch utilized.* Multiply the maltose figure by 0.9473. This is the factor derived from 324/342. (One gram of soluble starch, the amount present in 20 c.c. of starch solution, contains about 7.7 per cent of water making the starch used in the experiment equal to 0.923 gm. To estimate the true amount of starch utilized, multiply the above figure by 0.923.

## TO PREPARE A FIVE PER CENT GELATINE SOLUTION

Weigh out 50 grams of the highest grade culture media gelatine and add it to a liter of distilled water in a large beaker over a Bunsen burner. The temperature of the water should be kept below 60° C. to prevent scorching. Stir continuously until the gelatine solution is homogeneous. Pour carefully into a clean, previously heated, bottle. (Heated to prevent crack-

ing of the bottle.) Add toluol to cover the gelatine solution with one quarter inch surface. Keep at warm room temperature.

TO PREPARE A FIVE PER CENT SOLUBLE STARCH SOLUTION. (2)

Weigh out 50 grams of soluble starch. Stir into a smooth paste in a mortar, after adding slowly 50 c.c. of cold distilled water. Heat to boiling 950 c.c. of distilled water, add, with continuous stirring, the starch paste until a homogeneous mixture results. Pour carefully into a clean previously heated bottle and add toluol to cover to the depth of one quarter of an inch. Keep at room temperature.

TO PREPARE A TWENTY PER CENT OLIVE OIL EMULSION. (3)

Measure out thirty grams of powdered acacia, 60 c.c. of distilled water and 120 c.c. of the best grade of olive oil, stir the oil and the acacia into a smooth paste in a mortar. Always stir in one direction. When a thick homogeneous paste results, add quickly and in one amount 60 c.c. of water and continue stirring until a milk white emulsion is formed. Pour into a large graduate, add distilled water, with repeated washing of the mortar, until 600 c.c. of the emulsion is made. Add 1 c.c. of formalin as a preservative. Keep in the ice chest.

TO PREPARE AN ALCOHOL-ETHER SOLUTION. (4)

Take equal parts of 95 per cent alcohol and 40 per cent formalin, and mix. Make neutral with concentrated NaOH, that is, after adding a drop or two of phenolphthalein. Titrate the quantity to be used in the day's experiment to neutrality.

TO PREPARE AN ALCOHOL-ETHER SOLUTION. (5)

Five parts of neutral 95 per cent alcohol and one part of acid-free ether are mixed fresh for each day's experiments. Ten c.c. of this mixture made neutral with decinormal NaOH are used in each test.

TO PREPARE BENEDICT'S SUGAR REAGENT. (6)

Copper sulphate. (Crystallized) .....	18.0 gm.
Sodium carbonate. (Crystallized) .....	200.0 gm.
(One-half the weight of the anhydrous salt may be used.)	
Potassium thiocyanate .....	125.0 gm.
Sodium or potassium citrate .....	200.0 gm.
Potassium ferrocyanide. (5 per cent solution) .....	5.0 c.c.
Distilled water. qs. a.d. ....	1000.0 c.c.

With the aid of heat dissolve the carbonate, citrate and thiocyanate in enough water to make about 800 c.c. of the mixture and filter if necessary. Dissolve the copper sulphate in about 100 c.c. of distilled water and pour the solution slowly into the other liquid with constant stirring. Add the ferrocyanide solution, cool and dilute to exactly one liter. Of the various constituents the copper salt only need be weighed with extreme exactness. Twenty-five c.c. of the reagent are reduced by fifty grams of glucose.

To the majority of our readers it may, at first, seem as though we had not fulfilled our promise, that is that the method is not practical for the busy clinician, in that it requires too much time to perform. The author

here wishes to state that the time required to perform the entire set of determinations, including the calculation, is no more than one and one-half hours.

The reagents as we have shown are to be found in nearly all clinical laboratories and do not, when properly prepared, deteriorate on standing.

The accuracy of the method is best shown by the fact that out of five hundred or more determinations, all run in duplicate, we have yet to check outside the normal limits of error.

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### A STUDY OF THE YEASTS FOUND IN THE SPUTUM OF PATIENTS WITH ASTHMA AND CHRONIC BRONCHITIS\*

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THE description of fungi found in cases of bronchomycosis in the tropics has been considerably amplified by the studies of Castellani.<sup>1</sup> Among these fungi were recorded those of the genera *Monilia*, *Saccharomyces*, *Cryptococcus*, *Oidium*, *Aspergillus*, *Sporotrichum* and others. The elaborate classification of the *Monilia*, particularly by biochemical reactions has been especially applicable to the observations outlined in this paper. The occurrence of large amounts of yeast spores as well as vegetative forms in the sputum of patients living in Philadelphia and suffering from chronic bronchitis or asthma, suggested a similar study of these fungi. For this purpose the classification proposed by Castellani has been of value as a basis of comparison since the cultures here considered were of the genera *Monilia*, *Endomyces* and *Cryptococcus*.

In the white, sometimes chalk-like particles of the sputum of these patients, the spores were noted as fat-like globules varying in size from several to 10 or 15 microns in diameter. After washing in salt solution, cultures of these particles were made on Sabouraud's maltose agar or on lactose agar with a pH approximately 5.2. This reaction was conveniently obtained by adjusting the media with  $-\frac{N}{1}$  hydrochloric acid until it just turned brom-purple (dibromocresol-sulphonaphthalein) to yellow. Growth took place at 30° C. in one or two days. The cultures exhibited a white creamy unwrinkled appearance and a yeasty odor was detected in the cultures from the patients, S. B., A. S. and S. M. In the cultures classified as *Monilia*, budding yeast bodies were present and broad mycelial septate segments were noted in the bottom of the slant; no asci were seen. In those designated as being of the genus *Cryptococcus*, the culture consisted almost entirely of budding yeast forms with little or no attempt at mycelial formation. In

\*From the Clinical Laboratories of the Jewish Hospital, Philadelphia.

BIOCHEMICAL REACTIONS AFTER FIFTEEN DAYS' INCUBATION AT 35° C.

CULTURE	GENUS	LITMUS MILK	GELATIN	ARAB- INOSÉ	DEX- TRIN	DUL- CITOL.	GALAC- TOSE	GLU- COSE	INULIN	LAC- TOSE	LEVIT- LOSE	MAL- TOSE	MANNIT- TOL	RAPPEL- NOSE	SACCHA- ROSE	SAL- ICIN
S. B.	Monilia	ACD	0	A	0	0	A	AGs	0	A	AGs	A	A	0	A	0
V. M.	“	ACD	0	0	A	0	0	AG	AG	AGs	AG	AG	AG	As	AGs	0
J. K.	“	AC	0	0	0	0	AGs	AGs	AGs	AGs	A	0	0	0	AG	0
S. M.	Cryptococcus	AC	+	AA	A	0	A	AG	A	A	A	0	0	A	AG	A
I. L.	Endomyces	AC	0	0	0	0	A	A	0	A	A	A	AGs	0	AGs	A
I. G.	Cryptococcus	AC	0	AG	0	0	AG	AG	0	A	AG	0	AG	AG	AG	AG
A. S.	“	AC	0	A	0	0	A	AG	0	A	A	A	A	AG	A	A

Abbreviations in the table: A=acid; G=gas; s=slight; C=coagulation;  
 D=decolonized; + (gelatin)=liquefied;  
 AC=acid, coagulated, later alkaline,  
 alk



those grouped with the Endomyces, large numbers of spores were noted within mycelial elements and buds were given off at the sides; asci were present.

In the study of the fermentative reactions, the carbohydrates were made up in ten per cent solutions in distilled water and sterilized separately to avoid possible breaking up due to prolonged heating in the presence of bouillon. The ultimate concentration of the carbohydrates in the bouillon was one per cent. Brom-purple was used as an indicator. The results of these reactions are outlined in tabular form.

Intraperitoneal injections into guinea pigs of 2 c.c. of suspensions of these cultures in salt solution (approximately 50 million spores to 1 c.c.) after 24 and 48 hours, produced a slight exudate rich in eosinophilic cells and lymphocytes. Blood smears at the same time showed beginning eosinophilic granulation in many of the leucocytes, and also well developed eosinophiles. Eosinophiles were noted in great numbers in the sputum of the patients from which these cultures were derived and blood eosinophilia was a marked feature in these patients, ranging from 5 per cent to 30 per cent. It could not be said, however, that any constant proportion existed between the extent of the clinical and the experimental eosinophilia noted in the animals. This property of inducing eosinophilia is also noted in yeasts derived from other sources.

Demonstration of specific agglutinins by conducting macroscopic reactions with emulsions of spores and homologous sera did not yield conclusive results. However, in the case of S. M., the serum of the patient agglutinated the spores in dilution of 1 to 10, whereas control sera agglutinated only in dilution of 1 to 2. Complement-fixation reactions were inconclusive. The antigens were the water emulsions of cultures prepared after the methods outlined by Michel<sup>2</sup> in the study of *Monilia psilosis*. The antisheep hemolytic system was used in the test.

The patients from which these cultures were isolated did well when treated with iodides in the dosage of about 2 gms. a day or more. The injections of vaccines made from suspension of spores was given a tentative trial with fair results such as seen often with the use of bacterial vaccines. This procedure in a larger series would afford more definite information.

#### SUMMARY

1. Yeasts belonging to the genera *Monilia*, *Endomyces* and *Cryptococcus* were found in large numbers in the sputum of patients suffering from asthma and chronic bronchitis.
2. On the basis of biochemical reactions some of these are comparable to the yeasts, particularly *Monilia*, found in cases of bronchomycosis described by Castellani.
3. Production of eosinophilia in the peritoneal exudate and blood of guinea pigs after intraperitoneal injections was noted as a constant feature.

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## LABORATORY METHODS

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### ADAPTATION OF SHAFFER'S TITRATION METHOD FOR BLOOD SUGAR TO CLINICAL USE\*

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SHAFFER and Hartmann have recently published a complete system of methods for estimation of sugar in urine, blood, milk, and other solutions.<sup>1</sup> In all of these methods a copper reagent is used which is similar to Benedict's. This is partly reduced by the sugar. The further treatment of the mixture causes iodine to be set free. The iodine is titrated with thiosulphate, and from the amount of iodine estimated the copper equivalent of the sugar can be calculated.

Our interest was attracted to the micro-blood-sugar method because it enables physicians to make the estimations without the expense of investing in a reliable colorimeter.

Folin's method is the only colorimetric method of estimating blood sugar that we are willing to recommend. We find that the results secured by Shaffer's method are just as accurate as those by Folin's method.

In addition to strongly recommending Shaffer's method, we wish to propose a modification that removes the one difficulty which those who are not expert chemists would encounter in using the method. The difficulty referred to is that of standardizing the thiosulphate. We have overcome this satisfactorily by standardizing the thiosulphate against the copper reagent, which is very easily done. We have proved that this modification does not cause inaccuracy in estimation. Our experimental results will be reported after the description of the method.

*Preparing the Blood for Estimation.*—Draw into the syringe a strong solution of neutral potassium oxalate (30 grams of oxalate dissolved in 100 c.c. water), empty the syringe and expel the excess so that only a film of solution is left. Draw at least 3 c.c. of blood from a vein and empty the syringe into a dry tube. With an accurate pipette measure 2 c.c. of the blood into a dry flask and add 14 c.c. of distilled water. When the blood is completely laked add 2 c.c. of 10 per cent sodium tungstate solution and finally 2 c.c. of  $\frac{2}{3}$  normal sulphuric acid solution. The volume of the liquid is now exactly 20 c.c. Cork the flask, shake well and let it stand 5 to 10 minutes.

If all the protein has been precipitated, the mixture will be brownish-red

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\*From the Department of Biochemistry, University of Oregon Medical School, Portland, Ore.  
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in color instead of bright red, and only a trace of foam will appear on shaking. If the proper results have not been secured add 10 per cent sulphuric acid a drop at a time until satisfactory. (If more than 2 drops are required, the alkalinity of the stock solution of sodium tungstate must be reduced by treatment with a definite quantity of sulphuric acid.) Use small size filter paper for filtration. If the filtrate is not clear and colorless, return all the liquid (and precipitate) to the flask, treat it with more acid, shake well, and after a few minutes filter again. This method of freeing the blood of protein is exactly in accordance with Folin's<sup>2</sup> system of blood methods. What follows is Shaffer's special technic.

*Technic of the Estimation.*—Measure exactly 5 c.c. of filtrate into a large test tube and mix with it exactly 5 c.c. of the special micro-reagent. Plug the tube loosely with cotton and place it in a bath that is boiling actively. After exactly 15 minutes remove the tube and cool it at once under the tap. Then let it stand in a jar of cold water for 5 minutes. Add 5 c.c. of N/1 sulphuric acid, mix and let it stand 1 minute.

Titrate the liberated iodine by adding N 200 sodium thiosulphate as fast as drops can be counted (mixing well) until a distinct change is noticed, then add more slowly until the color becomes light yellow. Now add about 1 c.c. of 2 per cent starch solution and continue the titration cautiously but not too slowly until the characteristic starch-blue color is lost and only a pale copper-blue remains. When near the end-point determine the effect of each drop added.

*Calculation.*—In the special table (devised by us to correspond to our modification of Shaffer's method) the per cent of glucose in the original blood will be found opposite the c.c. of thiosulphate used for titration.

If the sugar content is greater than the highest in the table, dilute some of the blood filtrate with an equal volume of water and use 5 c.c. of this for the estimation, multiplying the results by 2.

#### REAGENTS\*

(1) *Microcopper Reagent.*—Dissolve each constituent separately.

(a) Dissolve 40 gm. C.P. anhydrous sodium carbonate (or 47 gm. pure photographic monohydrated carbonate) in 400 c.c. warm distilled water.

(b) Dissolve 5 gm. C. P. copper sulphate (crystals that have not effloresced) in about 100 c.c. water.

(c) Dissolve 7.5 gm. pure tartaric acid in 100 c.c. of water.

(d) Dissolve exactly 0.7 gm. pure potassium iodate in 100 c.c. of water.

(e) Dissolve 10 gm. pure potassium iodide in 100 c.c. of water.

(f) Dissolve 18.4 gm. pure neutral potassium oxalate in 100 c.c. of water.

When each is dissolved, mix (c) with (b), and pour the mixture slowly (with stirring) into (a). Combine (d), (e) and (f) and pour this at once into the carbonate-copper mixture. Transfer to a measuring flask. Rinse all the dissolving beakers with small portions of water. When cooled to room temperature fill to the liter mark and mix thoroughly. Keep the reagent in a

\*The Shaw Supply Co (of Portland, Seattle, and Tacoma) will supply any or all of the chemicals used for the method, including ready prepared solutions.

tightly corked bottle. A little sediment will be deposited, use the clear top liquid for estimations.

If the chemicals are pure, the microreagent run as a control will give the same titration whether heated in a bath 15 minutes or not heated. With each batch of reagent run one heated control and compare it with the unheated.

(2) *Standard Thiosulphate*.—Very dilute thiosulphate does not keep well. Prepare a stock solution of sodium thiosulphate a little stronger than deci-normal (dissolve about 26 gm. in about 1 liter of distilled water) and let it stand 2 days.

Dilute exactly 5 c.c. to 100 c.c. in a measuring flask. Mix and use this to titrate 5 c.c. of the copper reagent (after adding 5 c.c. water and 5 c.c. of N/1 sulphuric acid) following the directions given above for the rate of titra-

TABLE I

PER CENT GLUCOSE IN BLOOD CORRESPONDING TO C.C. THIOSULPHATE USED FOR TITRATION

C.C.	PER CENT	C.C.	PER CENT	C.C.	PER CENT	C.C.	PER CENT
18.6	.036	16.3	.101	14.0	.162	11.4	.232
18.5	.039	16.2	.104	13.9	.165	11.2	.237
18.4	.042	16.1	.106	13.8	.167	11.0	.242
18.3	.045	16.0	.109	13.7	.169	10.8	.247
18.2	.048	15.9	.111	13.6	.172	10.6	.252
18.1	.052	15.8	.114	13.5	.175	10.4	.257
18.0	.055	15.7	.116	13.4	.177	10.2	.263
17.9	.058	15.6	.119	13.3	.180	10.0	.268
17.8	.061	15.5	.122	13.2	.183	9.8	.273
17.7	.064	15.4	.124	13.1	.185	9.6	.279
17.6	.066	15.3	.127	13.0	.188	9.4	.285
17.5	.069	15.2	.129	12.9	.191	9.2	.289
17.4	.071	15.1	.132	12.8	.194	9.0	.294
17.3	.074	15.0	.134	12.7	.197	8.8	.298
17.2	.076	14.9	.137	12.6	.200	8.6	.303
17.1	.079	14.8	.140	12.5	.202	8.4	.307
17.0	.082	14.7	.143	12.4	.205	8.2	.312
16.9	.084	14.6	.146	12.3	.207	8.0	.316
16.8	.087	14.5	.149	12.2	.210	7.6	.325
16.7	.089	14.4	.151	12.1	.212	7.2	.335
16.6	.092	14.3	.154	12.0	.215	6.8	.345
16.5	.095	14.2	.157	11.8	.220	6.4	.357
16.4	.098	14.1	.159	11.6	.226	6.0	.368

tion. The titration will be less than 19.5 c.c. When duplicate titrations agree within 0.1 c.c. prepare a dilution of the thiosulphate such that exactly 19.5 c.c. will be required for the titration. For example, if the titration is 19 c.c. it will be necessary to dilute 5 c.c. of the stock solution to 102.6 c.c. (i.e.,

$\frac{19.5}{19.0} \times 100$ ). First dilute to 100 c.c. in a measuring flask then add the 2.6 c.c.

with a reliable pipette, and mix well. This ratio of dilution should be determined by similar titration of a control once in two weeks. Prepare the dilute solution each day that estimations are made. This dilute solution is approximately N/200, and is the one referred to in the technic. Keep the stock solution in a brown bottle well corked, set away where it will not get warm, under these conditions it does not deteriorate.

(3) *Starch Solution*.—This should be made once a week. We prefer



"soluble starch" but undoubtedly common corn starch could be used. Mix about 2 grams of starch with about 10 c.c. of water and pour it into about 90 c.c. of boiling water, mix and boil one minute. A few drops of toluol may be added as a preservative.

(4) *Standard Sulphuric Acid*.—The normal solution may be purchased if one is not used to checking standard solutions. For the  $\frac{2}{3}$  normal solution dilute 100 c.c. of the normal sulphuric acid with 50 c.c. of distilled water.

(5) *Sodium Tungstate Solution*.—C.P. tungstate should be used. The solution must be tested<sup>3</sup> for excess of carbonate as follows. Measure with a pipette 5 c.c. of the solution, add a drop of methyl orange solution and some distilled water, then titrate with the  $\frac{2}{3}$  normal acid until the yellow color changes to a slightly reddish yellow. Between 3 and 3.3 c.c. should be required. If the titration is greater add twice normal sulphuric acid (made by diluting about 11.5 c.c. C.P. acid to 200 c.c.) to the whole stock of 10 per cent sodium tungstate solution to bring its alkalinity down to the proper limits. Mix and titrate again.

TABLE II

COMPARISON OF ESTIMATIONS BY FOLIN'S AND SHAFFER'S METHODS							
	1	2	3	4	5	6	7
Blood							
Folin	.070	.109	.117	.124	.130	.167	.222
Reagent I	.069	.106	.118	.124	.132	.165	.222
Reagent II	.072	.109	.123	.126	.134	.169	.220
Reagent III	.069	.108	.117	.122	.132	.167	.219

Note: Control titrations of Shaffer's reagents were as follows:

I—19.55 c.c., II—19.3 c.c., III—19.7 c.c.

TABLE III

COMPARISON OF BLOOD SUGAR ESTIMATIONS BY SHAFFER'S ORIGINAL METHOD AND BY THE PROPOSED MODIFIED METHOD								
	1	2	3	4	5	6	7	8
Blood								
Shaffer	.047	.072	.099	.102	.120	.129	.229	.338
Reagent V	.048	.071	.100	.102	.122	.129	.230	.342
Reagent VI	.045	.069	.101	.103	.123	.130	.231	.341
Variation	.002	.003	.002	.001	.003	.001	.002	.003

Note: Reagent IV used for the original method gave a titration of 19.6 c.c. The control titration of reagent V used for the modified method was 19.9 c.c. and of reagent VI—19.0 c.c.

#### DISCUSSION

Different samples of copper reagent do not always give exactly the same titration value when accurate N/200 thiosulphate is used. We find that this value is generally close to 19.5 c.c., therefore, we have taken this figure as the average titration value. Varying the amount of iodate in the reagent changes the titration value.

Estimations by Shaffer's method using accurate reagents are practically identical with those by Folin's method (see Table II). Also when we used copper reagents that had a slightly different titration value (using exact N/200 thiosulphate the control estimation on reagent 2 is 19.3 c.c., and on

reagent 3 it is 19.7 c.c.) and made the thiosulphate correspond to the reagent in each case in accordance with our modification of the method. The results agreed very closely with the estimations both by Folin's method and by Shaffer's (with accurate reagents).

By changing the iodate content we secured copper reagents having a titration value differing more widely (19.0 c.c. and 19.9 c.c.). With these less accurate reagents we obtained very satisfactory results by using our modification of the method (see Table III). In most cases the variations from the accurate estimations by the original method were only 1 to 3 mg., and the greatest variation was 6 mg. Most of the variations were of the same order as were secured by running duplicate or triplicate estimations by any one of the methods. The Folin duplicates which we ran, differed by 1. to 2.3 mg. Shaffer triplicates varied by 0.5 to 4.5 mg. These results convince us that our modification does not perceptibly affect the accuracy of the Shaffer method.

The explanation for such good results with slightly inaccurate reagents is, undoubtedly, that the variation of the thiosulphate from a true N/200 solution compensates for the deviation of titration value of the copper reagent from the average value. For example, if the reagent is a little weak, then the thiosulphate solution prepared for use with it will be more dilute than N/200, thus avoiding the undertitration that would occur in a sugar estimation if an exact N/200 solution were used.

#### SUMMARY

An easy method of standardizing the thiosulphate is proposed as a modification of the Shaffer method, the thiosulphate being checked against the copper reagent.

Because of this adjustment of the solution the control estimation of the reagent is always 19.5 c.c. of dilute thiosulphate.

This constant titration value has enabled the authors to construct a table from which the per cent of glucose in the blood can be read without calculation.

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## AN AERATION APPARATUS FOR THE DETERMINATION OF UREA IN BLOOD\*

BY ARMADA T. WEATHERS, B.Sc., AND H. C. SWEANY, M.D., CHICAGO, ILL.

THE same difficulty mentioned by Boggs and McEllroy<sup>1</sup> in the distillation of ammonia formed by urease in the determination of urea in the blood by Folin and Wu<sup>2</sup> technic was also encountered in our work. A method similar to that described by Rakestraw<sup>3</sup> was then used with excellent results. As we perform a great many urea and nonprotein nitrogen determinations in this laboratory, we were compelled to devise a different means of separating the ammonia from the original digest than by the ordinary distillation method, where only one sample can be distilled at a time requiring the full time and

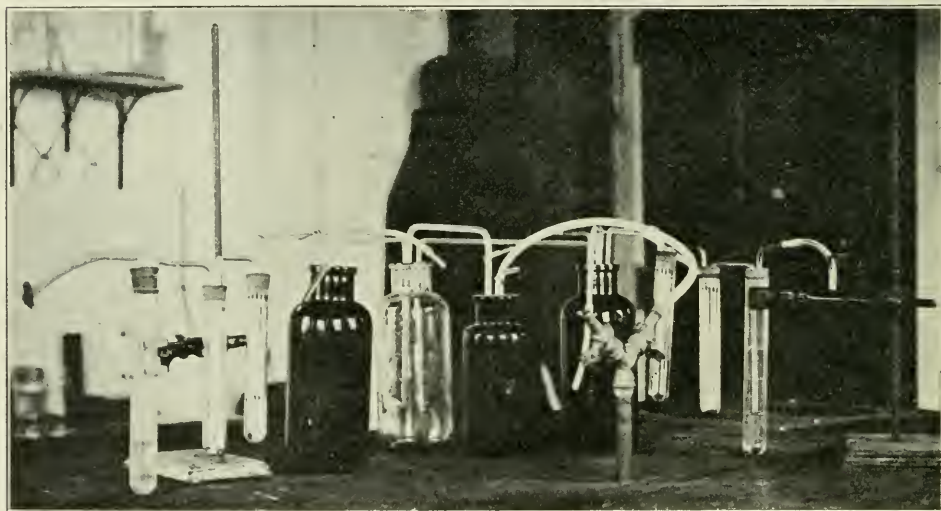


Fig. 1.

careful attention of one technician. The idea of designing an apparatus to run eight or more determinations simultaneously by aeration was decided upon in order to save this extra time and labor.

A very simple aeration apparatus was then devised. (Figs. 1 and 2.) It is operated by positive air pressure and requires practically no attention. This apparatus may be made from materials available in any hospital or student laboratory, viz.: test tubes, rubber stoppers, large, wide-mouth reagent bottles, glass, and rubber tubing and Hofmann screw elamps. The apparatus may be dismantled when not in use, does not require the cumber-

\*A Simple and Inexpensive Method whereby eight samples may be run at one time. From the Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Illinois.

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some pipes that are frequently used, and insures a dry ammonia-free air current for aeration. Fig. 3 shows the aeration unit. Four of these may be attached to bottle 3 and four to bottle 3' so that eight samples may be aspirated simultaneously. In Fig. 2, bottle 1 contains sulphuric acid, bottle 2 is full of glass beads and is used to catch any sulphuric acid that may come over. Bottles 3 and 3' contain cotton to break the air currents and to take out the last traces of the acid.

After the digestion of the blood filtrates with urease in ( $25 \times 150$ ) test tubes instead of ( $25 \times 200$ ) as described by Folin and Wu,<sup>2</sup> 2 drops of paraffine oil are added and the test tubes attached to the aeration units as in

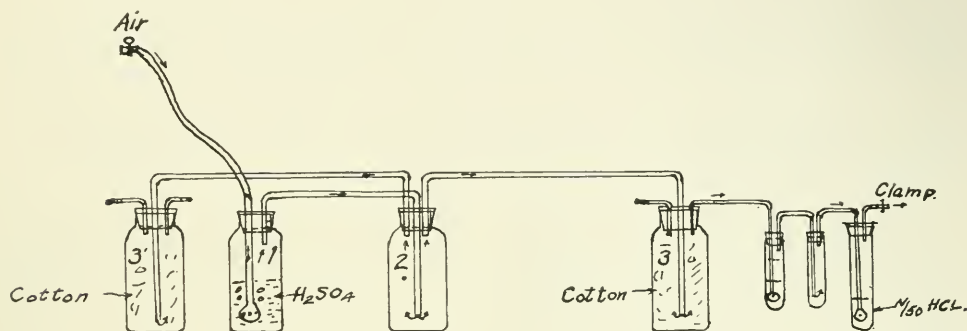


Fig. 2.

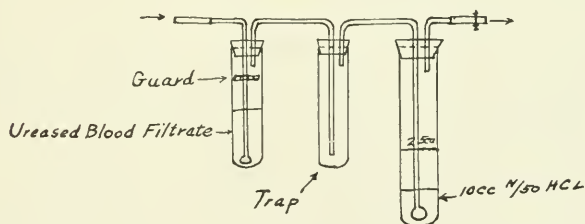


Fig. 3.

Fig. 3. Then 2 c.c. of 10 per cent NaOH are run through the tube, washed down with ammonia-free water and the unit connected to the apparatus. Thirty minutes is sufficient to drive over all the ammonia. During the first 15 minutes the air is passed slowly enough to give 2 bubbles per second. In the next 10 minutes the rate is increased so that 4 bubbles pass in the same time, and in the last 5 minutes the air is adjusted to give about 10 bubbles per second so that the last traces of ammonia may be swept out. Thus eight blood filtrates may be digested, aspirated, Nesslerized, and read in the colorimeter in one hour. Urea determinations on the same blood filtrates have been made by both the distillation and the aeration methods. The results of Table I indicate that a fair agreement between the two methods can be obtained.

This apparatus may also be used for nonprotein nitrogen determinations. It was found that the direct Nesslerization method of Folin and Wu<sup>2</sup> for nonprotein nitrogen gave cloudy solutions at times that could not be



TABLE I

CASE NO.	UREA N BY DISTILLATION	MG. PER 100 C.C. OF BLOOD			REMARKS
		UREA N BY AERATION	NONPROTEIN N.	AMINO- ACID N.	
15,817	9.95	10.00	26.2	7.0	Incipient T. B.
11,077	10.25	11.10	29.5	8.0	Far Advanced "
10,634	13.96	13.98	31.8	7.1	Incipient "
15,137	12.20	11.84	29.2	7.8	Far Advanced "
16,350	13.90	14.20	35.5	7.4	Incipient "
15,991	12.00	10.80	29.4	7.8	Far Advanced "
16,293	16.30	15.80	39.2	7.5	Far Advanced "
16,355	9.10	9.00	25.8	6.9	Incipient "
Sedg. 18123	11.14	10.20	26.9	8.0	

read in the colorimeter. As we run 15 to 40 determinations on each blood, there is usually no filtrate left to repeat the determination. So the Folin method was discarded and a microchemical Kjeldahl method used to decompose the organic matter in the Folin and Wu filtrate. The ammonia was set free with NaOH and aspirated as described above for urea.

This technic was then extended to the determination of ammonia, urea and total nitrogen in the urine. The ammonia is determined by Steele's<sup>4</sup> method up to the point of aeration, the urea by the method of Van Slyke and Cullen,<sup>5</sup> also up to the point of aeration, and the total nitrogen by the Bock and Benedict's method<sup>6</sup> up to the point of distillation. All three of these determinations may then be completed according to the aeration method described above.

The general use of this technic in routine work for microchemical nitrogen determinations, as a time- and labor-saving device, is due to the fact that many determinations can be made at the same time with practically no attention required during the process of aeration.

If the laboratory has no pressure line, a vacuum line or suction filter pump may be used. In this case, the aeration units must be reversed and the laboratory freed from all ammonia fumes. However, it is best to connect small wide mouth absorption bottles, containing sulphuric acid to each aeration unit. If it is desired to make more than eight determinations simultaneously, two more bottles containing cotton and designed as 3 and 3' may be connected to bottle 2. By this addition 16 determinations may be run at one time. In this case it is better to make at least 3 standards as the blood urea nitrogen should be Nesslerized as near the same time as the standard.

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## A STUDY OF THE AMMONIA PRESENT IN GASTRIC CONTENTS FOLLOWING TEST MEALS\*

BY JACOB ROSENBLOOM, M.D., PH.D., PITTSBURGH, PA.

### INTRODUCTION

CONSIDERABLE work has been published concerning the ammonia content of the gastric juice in the fasting state. On the other hand more data bearing on the ammonia content of the gastric juice in normal and diseased states is needed.

Bidder and Schmitt<sup>1</sup> in 1852 reported on the absence of ammonia from the gastric juice of man. Leo<sup>2</sup> noted a trace present in health and increased amounts in uremic cases. Rosenheim<sup>3</sup> discovered a trace of ammonia and thought it came from the gastric secretion, or from the decomposition of protein. Martins and Lütteke<sup>4</sup> found ammonia present only in cases of uremia. Ewald<sup>5</sup> found ammonia in 50 per cent of the cases examined. Strauss<sup>6</sup> found ammonia in eighty per cent of the cases he examined. Sticker<sup>7</sup> following test meals found ammonia in gastric contents, and thought it came from the saliva or from the decomposition of protein. Sommerfeld<sup>8</sup> could find no trace of ammonia in stomach contents of a man with a gastric fistula. Zunz<sup>9</sup> found small amounts of ammonia present and found it increased in cases of malignant disease of the stomach. Carlson,<sup>10</sup> in a man with a gastric fistula and in the contents removed by Rehfuess tube, found ammonia in all cases. The amount was from 2 to 3 mg. to each 100 c.c. of gastric juice of a man. Huber<sup>11</sup> found ammonia present in the gastric juice of a man in small amounts. It varied markedly in different individuals, and was increased by a high protein diet and by the addition of ammonium salts to the food. He also noted that the fundic mucosa contained the most ammonia, the pyloric next, and the cardiac end of the stomach contained the least and that the duodenal mucosa contained less ammonia than the fundic mucosa. He noted that in certain pathologic conditions especially gastric ulcer and cancer that the amount of ammonia in the gastric juice is definitely increased, but this increase may not be greater than that found continually in normal persons.

This paper contains a study of the ammonia content of the gastric contents removed one hour after an Ewald test meal, which consisted of two ounces of bread, and 200 c.c. of water. The ammonia was estimated by Folins<sup>12</sup> aeration method using  $n/50$  acid. The total acidity, free acidity, loosely combined acid, and the organic acid and acid salts were estimated by Töpfers method.

Table I contains the results obtained in the study.

\*Received for publication, February 26, 1923.

TABLE I  
SHOWING AMMONIA AND ACID CONTENT OF GASTRIC CONTENTS

CASE NO.	TOTAL ACIDITY	FREE ACID	LOOSELY COMBINED ACID	ORGANIC ACID AND ACID SALTS	AMMONIA	DIAGNOSIS
					MILLIGRAMS PER 100 C.C. CONTENTS	
1	40	20	10	10	0.6	Normal
2	50	18	20	12	2.1	"
3	60	20	—	—	4.6	"
4	46	18	—	—	0.9	"
5	50	20	—	—	2.0	"
6	48	18	—	—	3.5	"
7	45	16	—	—	3.0	"
8	55	22	—	—	2.5	"
9	50	18	—	—	3.6	"
10	46	24	—	—	5.6	Gastric ulcer
11	75	18	24	28	8.4	" "
12	38	0	14	24	1.4	Chronic appendicitis
13	70	0	50	20	1.9	" "
14	20	0	—	—	1.6	" "
15	54	—	—	—	1.5	" "
16	50	0	32	18	2.1	Gall stones
17	40	0	26	14	0.9	" "
18	24	0	—	—	4.2	Diabetes
19	46	24	18	4	1.9	"
20	130	50	55	25	1.8	Duodenal ulcer
21	72	28	26	18	1.4	" "
22	20	0	10	10	1.2	Cirrhosis of liver
23	65	30	25	10	1.1	Pyloric adhesions
24	68	50	13	5	1.4	Cholecystitis
25	55	25	25	5	4.8	"
26	52	28	10	14	3.5	"
27	30	15	10	5	7.0	Gastric cancer
28	66	15	30	20	0.8	" ulcer
29	15	0	10	5	1.5	Pernicious anemia
30	65	35	10	10	1.7	Secondary "
31	84	70	12	2	0.5	Duodenal ulcer
32	66	20	—	—	0.6	" "
33	84	32	—	—	1.2	Chronic appendicitis
34	75	20	—	—	1.5	" "
35	60	18	—	—	2.7	" "
36	80	22	—	—	3.0	Gastric ulcer
37	95	30	—	—	2.5	" "

## CONCLUSIONS

1. The ammonia content of the stomach contents following Ewald test meal varies from 0.6 mg. per 100 c.c. of contents to 4.6 mg.
2. There does not seem to be any diagnostic significance to the ammonia content of the gastric contents following a test meal.

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## AN UNSUSPECTED SOURCE OF ERROR IN THE GRAM STAIN\*

BY WM. M. SHEPPE, M.D., AND MARY G. CONSTABLE

### INTRODUCTION

**D**URING and immediately following the period of the World War, laboratory workers experienced great difficulties in obtaining reliable biological stains. This condition was especially manifested in the difficulty encountered in making satisfactory Gram stains.

Many private investigators, supplemented by committees of the American Society of Bacteriologists, studied numerous samples of American stains, particularly gentian violet, with the idea of determining their relative dependability.

Modifications of technic involving changes in staining times and types of decolorizers were advocated by Burke<sup>1</sup> and others. The former advised the addition of a pinch of sodium bicarbonate to the gentian violet on the slide but was unable to explain the increased reliability of the stain brought about by this simple procedure. This fact is of interest in view of our subsequent findings.

This laboratory was not exempt from the difficulties encountered elsewhere in making satisfactory Gram stains. Using the same stains and the same organisms, results varied with different students and with the same student from day to day. The chief difficulty lay in the too easy decolorization of Gram-positive organisms. Following the recommendations of the committee on the Descriptive Chart, several different samples of gentian and methyl violet were employed and the technic recommended by the committee rigidly adhered to. Some improvement was noted but annoying failures of Gram-positive organisms to retain the violet stain continued to occur and further search for the source of error was manifestly in order.

In considering the problem the authors were guided by the following facts:

1. Acids are very active in the removal of stains from bacteria.
2. The good results obtained by Burke after the addition of an alkali to the gentian violet may have been brought about by the neutralization of some unsuspected acid.
3. So far as we could determine, no investigation has been carried out on the properties or possible variations in chemical composition of the iodine solution.

Working from the above postulates a series of experiments was carried out along the following lines:

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\*From the Laboratory of Bacteriology and Pathology University of Virginia.  
Received for publication, April 27, 1923.



## PROCEDURE

I. Freshly made Lugol's solution, prepared according to the standard formula, was found to be neutral to litmus. This solution was used in the experiments listed under II and III.

II. Fifty c.c. of Lugol's solution were placed in each of four flasks and subjected to the following conditions:

Heat (Incubator 37° C.).

Heat and Moisture (Moist air at 37° C.).

Sunlight.

Cold (18° C.).

At the end of five days no change had occurred in any of the flasks.

At the end of ten days the following results were noted:

Flask No. 4 (exposed to cold) showed no change. The solutions in flasks 1, 2 and 3 were slightly acid to litmus. On the addition of a little sodium bicarbonate a definite evolution of gas took place. The color of the solutions was much lighter than when first prepared.

Smears were made from 24-hour agar cultures of *Staphylococcus aureus*. Gram stains were made on these smears using the standard technic but substituting the solutions from the flasks for the fresh Lugol's. Stains made using solutions from flasks 1, 2 and 3 were uniformly Gram negative. Flask 4 yielded a positive.

The above procedure was repeated three times, the results being the same each time.

III. To each of the four flasks of Lugol's solution, concentrated Hydriodic acid was added in the strengths of .25, .5, .75 and 1 per cent respectively. Smears were made and stained as above, except that the acidified Lugol's solution was added.

Results: No. 1 (.25 per cent) Organisms Gram positive.

No. 2 ( .5 per cent)           "           "           amphophile.

No. 3 (.75 per cent)           "           "           negative.

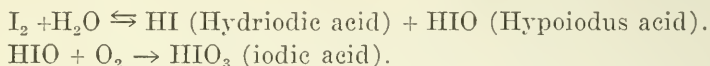
No. 4 ( 1 per cent)           "           "           negative.

IV. The Lugol's solution in use by a student who was obtaining false Gram negatives was obtained. Stains made with this solution were all Gram negative. The reaction of the solution was acid and the addition of a pinch of sodium bicarbonate resulted in a strong evolution of gas. Smears made with this neutralized solution were uniformly positive. This procedure was followed in the case of other students who were unable to make satisfactory stains and the difficulty in each instance was immediately overcome.

## DISCUSSION

The results of the investigation recorded in II show that the originally neutral Lugol's solution when exposed to the proper conditions is capable of undergoing a chemical change resulting in acid formation and that the amount of acid formed is quite capable of decolorizing Gram-positive organisms.

Bordier<sup>2</sup> has shown that this reaction takes place very readily when dilute solutions of iodine in water are exposed to light. The reaction is as follows:



Bordier believes that iodine does not form a true suspension in water but is in a colloidal suspension. The particles of iodine in suspension being in the colloidal state bear electrical charges and tend to act like large ions. Therefore the chemical properties of the electrically charged particles are not exactly identical with those of free iodine and hence will not combine with water to form the acid. However under the action of the violet and ultra-violet rays the electrical charges are removed from the ions. These particles thus discharged acquire their original chemical properties. They are then able to combine with the hydrogen of the water to form hydriodic acid.

The reaction, it will be noted, is a reversible one and this agrees with our experience as the solutions on further standing lost a part or all of their acidity. We think that this perhaps explains the variability of results obtained by a given student at different times.

The experiments recorded in III show that hydriodic acid is a powerful decolorizer of Gram-positive organisms acting in strengths as low as .5 per cent (pH 3). In IV the facts demonstrated are put to the practical test of experience and are found to satisfactorily correct the preexisting error.

It seems to us entirely possible that the good results obtained by Burke by adding an alkali to the gentian violet resulted from the neutralization of an acid Lugol's solution.

The light from individual desk lights, augmented by heat from steam sterilizers and bunsen burners is quite sufficient to inaugurate the reaction. The question arises at once, why is this discrepancy resulting from an acidification of Lugol's solution of a comparatively recent date? This we cannot answer conclusively but offer the following as possible explanations.

1. The old Grubler stains were possibly alkaline and so neutralized any acid formed in the iodine solutions.

2. Until about three years ago no artificial light was employed in the laboratory for microscopic work. The iodide solutions on the desks were therefore subjected to a very mild diffused daylight. With the introduction of the individual desk lights, the solutions were subjected to the action of the intense rays from the Mazda bulbs with which these lights are equipped. This light is not only of great intensity (which is probably of prime importance) but is rich in the light waves occurring toward the violet end of the spectrum. Bordier believes that these actinic rays are probably responsible for the inauguration of the reaction.

The second hypothesis is regarded as the most tenable explanation of the fact that the Lugol's solution in the laboratory was stable until the introduction of artificial light.

## SUMMARY

1. An error in the Gram-staining technic, resulting in the decolorization of Gram-positive organisms developed. This error was not corrected by changes in procedure or the use of other samples of gentian violet.

2. The Lugol's solution in use was found to have become acid. This acidity could be produced at will in test samples of the iodine solution by heat or light.

3. Stains made with these test solutions were regularly Gram negative as were those in which small amounts of hydriodic acid were added (.75 per cent).

4. Neutralization of the acidity by sodium bicarbonate resulted in the disappearance of false Gram-negative stains.

5. It is recommended that Lugol's solution be kept in dark bottles in a fairly cool place.

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## EDITORIALS

### *Insulin Therapy*

TOWARD the end of June, insulin was released for use by those physicians properly equipped with adequate hospital and laboratory facilities who are willing to assume the responsibility for its use. Prior to this a report was made in the *Journal of the American Medical Association* by the Insulin Committee, outlining the general procedure of treatment. Several independent articles have already appeared and an avalanche thereof may be anticipated. In the meantime a comprehensive symposium of articles has been published in the latest number of the *Journal of Metabolic Research*. It covers 438 pages and is an unusually valuable group of communications. Since the *Journal* is not available to the majority of physicians, it may be worth while to recapitulate the more important conclusions of the various authors.

*Route of Administration.*—The extract is as a rule given subcutaneously. An exception is found in severe acidosis and in coma, where the intravenous method is preferred. Joslin, Gray and Root gave insulin by mouth on two occasions when the patients had previously been rendered sugar free



by subcutaneous inoculation. Sugar rapidly reappeared in the urine and acidosis returned. Both disappeared upon return to subcutaneous administration. Woodyatt has given the drug through the mouth, rectum and vagina, intranasally and by inunctions. Positive effects were obtained only with subcutaneous and intravenous injections, while the results with other methods were either very weak, doubtful or frankly negative.

*Local Reaction.*—Early in the isolation of insulin the high salt content of the pancreatic extracts caused a stinging or burning at the site of injection, which persisted for an hour or more. This local reaction was rapidly eliminated but even now certain batches of extract have a similar effect lasting upwards of ten minutes. Some local tenderness follows treatment with occasional slight induration, but not as great as that following the hypodermic administration of many other drugs. Joslin reports that in over 5,100 injections no abscesses have developed. Induration has been fairly frequent and in one patient there developed a small crusted ulcer without evidence of infection which may have resulted from irritation of the tricesol. Wilder reports severe local injury with necrosis and sloughing of the skin in a few instances. One child developed a necrotic area ten by four centimeters on the thigh, which may be an unsuitable site for injection because of the tightness of the tissues. This also was attributed by dermatologic consultants to tricesol action.

Williams reports only one serious local reaction, which occurred with early extract before its purification had been perfected. He noted sterile abscesses in two others among several thousand injections. Fitz and the workers at the Brigham Hospital observed a local urticarial reaction in one instance, the extract used being one of the early isolations. Cutaneous tests with the protein of beef, pork and horse serum as well as with insulin failed to show any evidence of sensitization. Woodyatt avoids unnecessary pain, soreness, scarring and necrosis by using very small gauge needles (27-29) with a three-fourth to one inch length, inserted full length under the skin into the loose areolar tissue. He warms the solution before injection and takes particular care not to produce a lump during administration. Injections are usually given into the thigh. Allen and Sherrill have never had an infection or even a marked local reaction with the later extracts and remark on the fact that not a single infection has occurred among the patients giving their own injections at home.

*General Reaction.*—The most important general reaction is the development of hypoglycemia. This will be discussed under a separate heading. Joslin and his coworkers observed protein reactions in four patients: Williams has seen two anaphylactic reactions, and Wilder three. Geyelin observed serum sickness in three of nine cases and found it necessary to desensitize two with small doses of insulin. These reactions were all observed before the extract was more successfully purified and none have been reported from the later products.

*Frequency of Administration.*—Banting, Campbell and Fletcher have found that in the average case the injections need not be given three times

a day. When the insulin requirement is small it may be taken in one dose before a meal in which the greater part of the total carbohydrate has been concentrated. This does not maintain a low blood sugar level. As a rule, two doses daily, one before breakfast and a second before the evening meal, are preferable. The dividing of doses in this way allows a greater utilization of food stuffs per unit of insulin.

Joslin, Gray and Root feel that it is as yet too early to state definitely whether two injections daily would be as efficacious as one given before each meal. They recognize the advantages from the patient's viewpoint of administering the drug but twice daily. Williams remarks "the time and frequency of dosage are important. Apparently the action of extracts lasts only a few hours. In severe cases it should be administered twice or better three times a day in properly spaced doses." Wilder and his associates believe that maximal results were obtained with single injections of the entire amount thirty minutes before breakfast, and with food distributed equally between the three meals. This was particularly so when the dosage did not exceed thirty insulin units. As a rule, the second meal comes early enough to neutralize any severe late effect on the glycemie level. The danger of hypoglycemic reaction may be minimized by careful balancing of the diet, and should symptoms arise, by prompt administration of small amounts of sugar. They favor a single daily injection not only because of the greater ease in treatment, but also because individual cases who were treated by both methods have appeared to do better with single injections.

Woodyatt has in his work practically eliminated multiple daily doses and in ninety per cent of all cases, administers a single morning injection. Rarely he gives two doses a day. This of course does not apply to complications such as acidosis or coma.

Allen and Sherrill prefer divided doses with two or three per day, even when the dosage has been as low as one and two units per day. In the severe types they prefer three injections. Occasionally with three daily administrations, the blood sugar level remains satisfactorily low during the day but rises in the night so that by morning glycosuria exists. Here they prefer to give the extract four times daily at six hour intervals with the feedings appropriately arranged.

*Dosage.*—The average unit is considered as metabolizing 1-1.5 grams of glucose. Woodyatt has seen cases in which one unit reduced glycosuria by as much as three grams. In mild arteriosclerotics and renal diabetics on the contrary, insulin exerted little effect. The ratio varies in practical work with the individual patient and particularly with the extent of glucose retention. Thus the value of each unit per gram of exogenous glucose is higher after the patient has been thoroughly treated than at the beginning when endogenous stores were present in excess. However, for the starting of treatment, the above ratio may be safely accepted. If after one or two days of observation a patient has been found to excrete forty grams of glucose in twenty-four hours, twenty-five units of insulin may be prescribed for the first day's dose.

All writers stress the importance of individualized dosage and the avoidance of hypoglycemia. We shall see that there is some divergence of opinion regarding the necessity for maintaining complete aglycosuria. All have found that in acidosis or coma, or with superimposed infections, the dose required to render a patient sugar free and to remove the hyperglycemia is greater than without such complications. When at the beginning of treatment the blood sugar is high and there is a consequent larger concentration in the tissues, larger quantities of insulin are required to render the patient sugar free than is later necessary to keep him so. Thus in one case after four days, insulin was reduced from sixty to forty units, the total glucose intake remaining the same. Joslin, Gray and Root give the average daily number of units in fifty-three patients as eleven. They find no evidence at present that the dosage must be increased provided we disregard an increase early in treatment, to care for the constantly increasing diet, and allow for increased metabolic needs after the undernutrition has been relieved. Wilder and his coworkers vary the dose according to the patient's basal tolerance and with the diet. In general they provide a food allowance of 2000 to 2600 calories. As a rule between ten and thirty insulin units will be needed to raise the tolerance sufficiently to permit this desired higher caloric value. They give as high as thirty units in one dose.

Those working at the Presbyterian Hospital in juvenile diabetes have given somewhat larger doses with higher total caloric intakes, so that the children may gain weight more rapidly. Woodyatt has developed a relatively simple system of dosage determination. On a basal diet he establishes a daily excretion of sugar. Thus if the individual excretes steadily twenty-five grams of glucose, twenty units will about render him sugar free. He then receives one-half of this calculated dose. This dose is increased by five units daily, and later the caloric intake is gradually increased to the desired amount. Allen and Sherrill state that the dosage for regular use ranges between four and seventy units per day. Under any plan compatible with fair strength and comfort, it is seldom possible to treat a really severe case with less than twenty units per day. Several of Allen's cases received between thirty and fifty units while a maximum of seventy units was reached in only one case.

*Hypoglycemia.*—The reaction of greatest importance following insulin results from too rapid or too great fall in blood sugar level with resulting hypoglycemia. Fletcher and Campbell found that a low level was reached anywhere from two to twelve hours after a single administration. The glycosuria threshold is usually around 170 mg. per 100 c.c., but has been observed as high as 300 and as low as 100. The higher the initial blood sugar readings the more rapidly does the percentage tend to fall. There is no definite correlation in all cases between the amount of insulin administered and the degree of fall even though the initial blood sugar be the same. In diabetes the blood sugar is easily lowered to the normal range but is somewhat less easily reduced below this.

The symptoms of hypoglycemia consist of excessive hunger, nervousness,



tremulousness with sometimes actual tremor, and at times, a feeling of weakness or a sense of goneness. In children the pulse may increase while in adults sweating becomes an outstanding feature in the more profound hypoglycemias. As the condition progresses the nervousness may become definite anxiety, excitement or even emotional upset. Syncope or collapse may occur but convulsions have not been observed.

Some patients become aware of hypoglycemia when the blood sugar is between .08 and .09 per cent. Others experience no symptoms with levels as low as .054. A severe reaction has been observed with a blood sugar percentage of .060 per cent and again a mild one in an individual with a concentration of .040 per cent. A blood sugar percentage of .035 is usually accompanied by unconsciousness. Reactions are generally more severe and more prolonged in the extremely undernourished. Even when there has been an accurate balancing of insulin and carbohydrate intake, reactions occasionally occur, due presumably to some unusual delay in the absorption of food. Reaction usually occurs two hours after administration but may be delayed, and should be particularly avoided at night when the patient may be asleep. Joslin observed thirty reactions in the course of 5,153 injections. These occurred in eleven individuals. The smallest dose causing hypoglycemic symptoms was one insulin unit. This occurred in a man with tolerance for 114 grams of carbohydrate, but who was weakened by diarrhea. Allen has observed a reaction after one-half unit in an individual extremely weakened and emaciated, on a low diet. He observes that heavy exercise increases the tendency to hypoglycemia.

The Toronto observers particularly found that reactions were prone to occur when changing to a new batch of insulin, the unit value of which might be slightly greater than that which had been previously used by the patient. This variation in the strength of batches has been practically overcome by Eli Lilly and Company. Woodyatt and Wilder standardize their new batches of insulin by clinical tests on appropriate patients.

Treatment consists in the administration of glucose usually by mouth. Orange juice is very successful. Other sugars than glucose are not as highly recommended. If necessary, glucose may be administered intravenously. Other substances such as hot tea or coffee and beef extracts produce temporary relief from symptoms but no increase in blood sugar concentration and are invariably followed by relapse. As a rule there is no recurrence after the administration of glucose. Often five grams of glucose will tide a patient along till the next meal. Twenty grams are frequently administered.

The administration of epinephrin is recommended in unconsciousness. This apparently causes a mobilization of tissue sugars into the blood. Such treatment must be followed by the giving of orange juice or some preparation containing a moderate quantity of dextrose. Allen reports one unusually severe case who did not respond to epinephrin but who revived immediately following an intravenous injection of glucose. The possible interpretation was that the carbohydrate stores in the tissues had already been too thoroughly exhausted. Allen has found that in emergency, sterilization



for intravenous glucose administration is not indispensable, for a clean filtered solution injected intravenously does not cause infection.

The Toronto workers advise that each patient experience a mild hypoglycemic reaction while still under hospital observation so that he may more readily recognize the symptoms should they occur under home treatment.

*Acidosis and Coma.*—In impending coma and actual coma the amount of insulin administered must be decidedly higher than in the less severe cases. The drug may be given intravenously and if large doses are administered, glucose should be given at the same time. Ketone bodies appear to be actually burned or synthesized into some nontoxic body, possibly carbohydrate in nature. At all events they are disposed of in the presence of large amounts of insulin without corresponding changes in the urine or expired air. Not all coma cases recover with insulin, probably because of permanent toxic damage to the nervous system or other tissues. If this be true early administration is essential and the intravenous method is preferable. In some of the earlier cases such as two reported by Williams, death was due to insufficient insulin. The majority of those who have progressed to a fatal outcome were suffering from complicating conditions such as influenzal pneumonia, moist gangrene, etc. Carbohydrate administration prevents hypoglycemia, furnishes energy, reduces the incomplete combustion of fat and protein, thus limiting ketone production, and finally, aids in the combustion of those ketones already present in the blood and tissues.

Allen and Sherrill outline a plan of treatment to be followed in precoma. In actual or threatened coma a blood sample is taken and 25 units of insulin are injected into the vein through the same needle. An additional dose of from 25 to 50 units is immediately given subcutaneously. Further subcutaneous doses follow at intervals of one or more hours, depending upon the laboratory findings. Glucose is given simultaneously so that the blood sugar concentration may be maintained above 300 mg. per 100 c.c., while the acid bodies are being cleared up as rapidly as possible. At least 100 units are usually required in a dangerous coma case. Other authorities repeat the intravenous dosages.

Death in coma under insulin has been variously ascribed to hypoglycemia, infection, and unknown toxic properties of the drug. There is some evidence that extremely high dosages of the latter do possess some toxic action. More probably death in unsuccessful cases is due to irreversible protoplasmic injuries which result from acidosis, but are not removed by removal of the latter.

*Administration of Alkali.*—Alkali therapy when used in conjunction with insulin appears to have considerable value in coma. To be sure, excess of sodium bicarbonate does harm and this accounts for the firm stand of a number of clinicians against its use. The Toronto observers found that while insulin diminished the extent of acidosis to a remarkable degree, the alkali reserve remained at a very low level in spite of reduction in blood acetone bodies. If acidosis has been overcome, alkali may therefore be administered to cause a return to normal of the alkali concentration in the blood, with

definite clinical improvement. Alkali should be given as early as possible. Since the absorption of substances introduced into the stomach during coma is most erratic, intravenous administration is preferable. Campbell suggests from the work of Palmer and Van Slyke that one might safely introduce 20 grams of sodium bicarbonate per 84 pounds body weight.

Woodyatt in treating precoma institutes a diet containing 0.5 to 0.75 grams protein and 2.0 to 2.5 grams fat per kilogram with a low carbohydrate ratio and gives the patient at once 20 grams of sodium bicarbonate by mouth. The base is repeated at the rate of 15 to 20 grams per hour until the blood plasma shows a reading of 40 to 45 volumes per cent  $\text{CO}_2$ . When insulin is used effectively he usually discontinues the soda after giving from 20 to 60 grams. Allen and Sherrill remark that any signs of acidosis should call for complete exclusion of fat and practically a diet of carbohydrate alone. They recommend sodium bicarbonate in five gram doses by mouth up to 20 to 50 grams in 24 hours (rarely in small doses intravenously).

*Complicating Infections.*—Opinion is unanimous that the high nutritive level made possible by insulin and the decrease in hyperglycemia are of decided benefit in complicating infections. Diabetes with tuberculosis may now be treated with more hope of success. The results with other medical and surgical infections, particularly gangrene, have been fairly satisfactory. The general resistance and the healing of wounds appear to be no better under insulin than they have been heretofore under efficient dietary control. However the results are decidedly better than when dietary control was not complete and benefit is particularly seen in the complicating acidosis. The lowering of tolerance which customarily occurs in infection may be combated by increase in the insulin dosage. Joslin remarks that general infections were uninfluenced by insulin save that complicating acidosis was lowered. Local infections appeared to heal more rapidly.

*Optimal Diet.*—All observers are agreed that the diet even under insulin should, particularly in adults, be no more than sufficient for the patient's metabolic needs. No attempt should be made to fatten patients needlessly. The diet theoretically may be raised to any desired amount, provided sufficient insulin be administered and provided the proper balance of protein, fat and carbohydrate be maintained. The diet should not be increased beyond such levels as will satisfy reasonable normal demands for energy. There are several reasons for this. Increased calorie intake causes increase of metabolic rate. Increased intake necessitates more insulin and more frequent injections with consequent greater liability towards hyperglycemia and also towards hypoglycemic reactions. Furthermore, if the patient who has attained a certain degree of obesity suddenly finds himself unable to procure his insulin, there is great danger of resultant acidosis. Extreme undernutrition is no longer necessary but moderate undernutrition still appears preferable to hypernutrition. The observers at the Mayo clinic aim to provide in general a food allowance of about 2,000 to 2,600 calories.

Williams records that the feeding of high diets and attempts to make their utilization possible by large amounts of insulin has not been satisfactory.

The best treatment apparently is a diet which will enable the patient to meet the energy requirements of light work with sufficient insulin to insure the metabolism of the food. Geyelin, working with children has attempted to bring the weight up to the normal for their age and height. The gain in weight ranged from two pounds in three months to 22 pounds in seven months. There was a corresponding increase in muscular strength and vigor and great improvement in the patient's sense of mental and physical well being.

Woodyatt in determining the practical limit of the caloric intake, considers the patient's financial status and his ability to pay for large amounts of insulin, the size of the patient and the nature of his life and work. One man may be able to attend to his daily duties with 2000 calories while a larger man doing heavier work may require more. From the economic viewpoint a certain individual may be able to do more efficient work with larger doses, thereby increasing his earning capacity.

Allen and Sherrill call attention to the increased danger of death from coma in patients receiving a high caloric and high fat intake. In children they provide for the demands of growth and development. If a higher diet raises the earning capacity, a higher dosage of insulin may be a good investment even for a day laborer. Accuracy of control is another factor. Thus a child of wealthy parents who will always be attended by a specially trained, highly skilled nurse, is fed more abundantly than another child treated on a charity basis who will ultimately have to carry the responsibility of his own treatment at home. They emphasize particularly that attention to the diet is more important when insulin is being given than otherwise. "All our attempts with lax or inaccurate diets in conjunction with insulin treatment have led to disastrous results. Insulin has tremendously improved diabetic treatment but has not simplified it."

*Effect on Basal Metabolism.*—Wilder, Boothby and their coworkers found in certain cases that the basal respiratory quotients and the basal metabolic rates were but little affected by insulin. Fitz, Murphy and Grant, who made a special study of the effect on metabolism, confirmed the conclusions of Banting, Best and their associates in that insulin caused a definite increase in the respiratory quotient. Sometimes the quotients remained up as long as three days after the drug had been discontinued, suggesting that the effect of insulin does not immediately wear off. Joslin, Gray and Root found the basal metabolism increased nine per cent after prolonged treatment in a series of eleven patients. The respiratory quotient rose with food after insulin to above unity.

*Effect on Protein and Fat Metabolism.*—Banting, Campbell and Fletcher have shown a definite influence of insulin in sparing nitrogen. Fitz, Murphy and Grant found an apparent influence on protein katabolism, enabling the diabetic to store nitrogen instead of excreting an amount equal to or greater than that ingested. Insulin evidently spares proteins. Wilder, Boothby and their coworkers found that insulin had less effect with a high protein, low fat diet, than with a relatively higher fat and lower protein diet, the caloric



content and carbohydrate content of each diet being the same. Allen and Sherrill on the other hand, report that these differences are greatest in short tests and in the long run are not sufficient to warrant the inconvenience and discomfort, if not actual danger, of an overbalanced ratio. They follow no fixed rules of protein requirement or ketogenic-antiketogenic balance, but content themselves with giving a safe sufficiency of protein and carbohydrate. They commonly allow in a diet of 2000 calories, 100 grams protein and 100 to 150 grams carbohydrate. The difference in the insulin requirement as compared with a diet composed more largely of fat is relatively slight in the long run, while there are important differences in comfort, in the ability to eat articles customarily found in the normal diet, and in freedom from acidosis dangers.

*Must the Patient Be Maintained "Sugar Free"?*—There appears some diversity of opinion among the various observers as to whether for best results the patient's diet and insulin dosage should be so regulated that he remains entirely free from glycosuria. There is also divergence of opinion as to how near to normal the blood sugar should be maintained. Banting, Campbell and Fletcher remark that blood sugars well above normal provide some protection against the occurrence of hypoglycemia and also allow greater economy in the use of insulin, but that it hardly seems justifiable to abandon as a principle in treatment the maintenance of the blood sugar at a normal level. Hyperglycemia may be a factor in some of the degenerative processes such as arteriosclerosis, optic neuritis and so on, and besides blood sugar above the normal level must throw a greater load upon the islet tissue of the pancreas. They attempt to keep the patient sugar free.

Joslin, Gray and Root brought an average fasting blood sugar of 0.24 per cent down to 0.19 per cent at the end of treatment. They also attempt to keep the patient sugar free. Geyelin has purposely never totally abolished glycosuria for any successive period longer than ten days. He and his associates allow a moderate glycosuria in all cases as the most practical method of lessening the possibility of insulin overdosage. They attempt to maintain the glycosuria at ten grams or less per day. Woodyatt finds that owing to unavoidable variations in diet, insulin dosage, exercise, etc., the old cases with low fixed tolerance limits are kept sugar free with difficulty, and he believes it unnecessary to keep this type sugar free because they have little or no natural tolerance to lose. On the other hand, all those having a considerable natural tolerance or power to regain such should be kept sugar free if possible.

Allen and Sherrill favor maintaining the urine free from sugar. They point out that the loading of urine and blood with sugar is decidedly abnormal and that the remote effects thereof will be bad. If thorough treatment is preferable in the emergency conditions it is difficult to see why it should not be equally advantageous before their development. They call attention to the lowered resistance to infection, arteriosclerosis, gangrene, etc., which may follow hyperglycemia, and insist that lax treatment with insulin does no more than convert the case into a milder but still active form of diabetes.



They find it feasible for patients who have received proper instruction to remain free from glycosuria and nearly free from hyperglycemia on their return home.

*Does Insulin Produce a Permanent Increase of Tolerance?*—Uppermost in the minds of all since the advent of insulin has been the question—"will its administration produce permanent cure?" This has been the hope of all sufferers from the disease. Banting, Campbell and Fletcher feel at present when no case has been under continuous treatment for as long as a year, that it is too early to prognosticate remote results. They find that such evidence as we have indicates that the disease may be satisfactorily controlled even after the patients have left the hospital. They do not claim definite permanent improvement in sugar tolerance. Some cases have, however, given rather suggestive results. They have estimated the carbohydrate utilization power after insulin in cases who had previously been under prolonged dietary treatment. They find that certain patients do regain some increased power of carbohydrate utilization under insulin therapy. Increases in total glucose tolerance of as much as twenty to thirty grams have several times been observed. This increase persists as long as excessive demands are not made upon the pancreas by excessively high diet or high blood sugar levels. In other patients no increase in carbohydrate tolerance has been obtained.

Joslin, Gray and Root do not accept entirely the conclusion of Allen and Sherrill that diabetes is a nonprogressive disease. They are not sure that even under insulin therapy it is not a progressive disease. Thus Miss N. after several months' treatment, has rather more difficulty in keeping free from glycosuria with 20 units daily than she had formerly. They stress that at the beginning of treatment and until a balance is obtained, more insulin will be required, but that the subsequent decrease to a smaller amount does not indicate an increased tolerance. This latter level of insulin dosage cannot be further decreased.

They emphasize that the diabetic on dietary treatment alone, the patient with subnormal metabolism, has a different type of disease and cannot be compared in all features with a diabetic receiving insulin, whose metabolism is nearly normal. They caution against the production of the diabetic with increased metabolism. Slight increases in insulin requirement early in treatment may be explained by the resulting increase in metabolism rather than diminution on the power of insulin to act.

Williams in a group of severe cases found it necessary to practically double the amount of extract required when the treatment was first instituted. In each case, however, there resulted a gain in weight of from 15 to 25 pounds and an even greater gain in strength. In three cases there appeared to be considerable gain in ability to utilize glucose. In several other cases treated for shorter periods of time and notably in children, it was found necessary to gradually increase the dose from 20 to 50 per cent. This suggests a gradual failure in pancreatic function or response to the drug. None of his 43 cases showed evidence of increased pancreatic func-

tion. A gradual decline in function indicates the necessity for continuance of careful dieting.

Allen and Sherrill point out that gain in weight naturally calls for an increase in insulin dosage. Three diabetics seemed to lose tolerance demonstrably when glycosuria was permitted along with insulin treatment. Several others have shown gain of assimilative power with insulin beyond anything that could be expected from dietary treatment alone.

*Renal Diabetes.*—Doses of five and ten units of insulin have exerted little effect on the glycosuria in several cases examined by Woodyatt. He does not state definitely regarding the blood sugar.

*Conclusions.*—The researches so far reported indicate that insulin supplies in its entirety whatever metabolic deficiency the diabetic patient possesses. This is an important point in evidence for those who contend that diabetes is primarily and entirely due to disturbed pancreatic function. Its use revolutionizes the treatment of diabetes but it does not simplify it. Its abuse may jeopardize the patient. It should not be used to fatten individuals unnecessarily nor to enable an obese diabetic to remain obese. The drug is powerful both in its beneficial and in its harmful effects and should only be used with a full understanding of both.

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 Fletcher and Campbell: Jour. Metabolic Research, ii, 637.  
 Joslin: Jour. Metabolic Research, ii, 651.  
 Wilder, Boothby, Barborka, Kitchen and Adams: Jour. Metabolic Research, ii, 701.  
 Williams: Jour. Metabolic Research, ii, 729.  
 Fitz, Murphy and Grant: Jour. Metabolic Research, ii, 749.  
 Geyelin, Harrop, Murray and Corwin: Jour. Metabolic Research, ii, 767.  
 Woodyatt: Jour. Metabolic Research, ii, 793.  
 Allen and Sherrill: Jour. Metabolic Research, ii, 803.

—W. T. V.

## BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

### *Standard Methods of Milk Analysis\**

THE American Public Health Association and the Association of Official Agricultural Chemists established in 1922 a series of standard methods of milk analysis, including bacteriological and chemical examination and methods for the detection of preservatives, coloring matters, etc. These standard methods were published in a pamphlet of forty pages, which is now in its fourth edition.

\*Standard Methods of Milk Analysis. Of the American Public Health Association and the Association of Official Agricultural Chemists. Fourth edition. Paper. Price forty cents. 1 p. 40. American Public Health Association, New York, 1923.

*Practical Physics\**

THE feature which immediately appeals in this book is the successful attempt made by the author to so arrange physical laboratory experiments that a minimum of special apparatus is necessary. In fact a great proportion of the experiments may be carried out with no other equipment than such articles as those with which we come in daily contact. Such for instance is an experiment to determine the area of an irregular figure by means of squared paper, or an experiment to determine the circumference of a cylinder by multiple windings of an ordinary thread. The work fulfills the title of the book in that it is practical in every sense and at all times keeps before the student a realization that physics is primarily not a matter of expensive laboratory equipment, but is a phase of the every day problems of life.

The volume is a laboratory manual rather than a text book and is so arranged as to promote interest in the work and individuality in solving problems.

*The Physiology of Twinning†*

THE PHYSIOLOGY OF TWINNING, dealing primarily with the causes and consequences of twinning, is an elaboration of an earlier volume entitled *The Biology of Twins*, published in 1917. The book serves as a vehicle for the exposition of the author's theories regarding the production of twins and monsters in general. Much of the discussion is devoted to consideration of the comparative physiology or pathology, as one may choose to call it, of twinning. In it is an able résumé of the author's studies and of the studies of others on the phenomenon in the starfish, the earthworm, in fishes, birds, amphibia, reptiles, armadillos, and in mammalia particularly human beings.

Early in the book the author summarizes his theory which is briefly that twinning is a phenomenon of the physiologic isolation of equivalent parts of the blastoderm and regulation of the isolated or twinned regions into complete embryos. The cause of the physiologic isolation may be a temporary cessation or radical retardation of development at a critical period, such that the polarity of the embryo becomes weakened and there are no longer high and low metabolic regions. All parts of the embryo are left on a parity. When normal developmental conditions return, some new part of the embryo becomes the apical point for the establishment for a new or additional gradient. There thus develop two or more growing points or axes of growth. The application of existing facts to this theory is developed throughout the remainder of the volume. The work and theories of others are given due recognition.

\**Practical Physics*. By J. A. Crowther, Sc.D., F. Inst. P., sometime Fellow of St. John's College, Cambridge; Demonstrator of Physics in the Cavendish Laboratory; University Lecturer in Physics as applied to Medical Radiology, Cambridge. Cloth. Price, \$3.25. Pp. 260. Henry Frowde and Hodder & Stroughton, London. 1922.

†*The Physiology of Twinning*. By Horatio Hackett Newman, Professor of Zoology, University of Chicago. Cloth. Pp. 230. The University of Chicago Press, Illinois, Price, \$1.85. 1923.



*Ortner on Pain\**

A RATHER extensive treatise by this distinguished Viennese physician has recently appeared in an English translation. It consists of two volumes, the first devoted to a discussion of abdominal pain, the second, although entitled *Generalized Pain*, dealing chiefly with regional pain originating in portions of the body other than the abdomen. Many volumes have been written on the single symptom pain and it has been dealt with by as many methods. From the breadth of his own clinical experience, Ortner is able to enumerate practically all of the known causes of pain referable to each region of the body. This he does in a didactic manner. Having enumerated the possible causes, he gives scant attention, as a rule, to true differential diagnosis.

The two volumes have not been translated by the same individual. That on abdominal pain is easy reading and an excellent translation. That on generalized pain, whatever its intrinsic value in the original, has lost much thereof in the translation.

*The Chemistry of Tuberculosis†*

THE collaborators responsible for this volume have devoted many years to study of the chemistry of the tubercle bacillus and its environment. By environment, we refer not only to changes in artificial culture media which influence or are influenced by the growth of the tubercle bacillus, but also to reactionary changes in the living host. They have had occasion to review in this connection a tremendous mass of literature. From this they have sorted the work of undoubted value, and the present volume stands as an unusually complete compilation thereof. The subject matter treated would at first appear to interest but a few individuals, chiefly research students in the field of tuberculosis. Nevertheless the work is excellently done and at many points touches on practical features in the study of clinical tuberculosis. It will without doubt be of practical value to all interested in tuberculosis, whether from a clinical or a research point of view. The literature on each phase of the chemistry of tuberculosis is reviewed impersonally. At the close of each chapter, there appears a recapitulation in which the authors have permitted themselves some critical discussion, which enhances the value of the treatise. The chapter on Specific Chemotherapy is of particular interest and the conclusions are thoroughly conservative.

\*Abdominal Pain. By Prof. Dr. Norbert Ortner, Chief of the Second Medical Clinic at the University of Vienna. Authorized Translation by William A. Brams, M.D. Formerly Lieutenant-Commander, Medical Corps, U.S.N. and Dr. Alfred P. Luger, First Assistant, Second Medical Clinic, University of Vienna. Cloth. Price \$4.00. Pp. 362. Rebman Company, New York, 1922.

Generalized Pain. By Prof. Dr. Norbert Ortner, Vienna. Only authorized Translation into the English Language of the 2nd German edition by Francis J. Rebman. With an introduction by Thomas Webster Edgar, M.D. Cloth. Price, \$4.00. Pp. 596. Medical Art Agency, New York, 1922.

†The Chemistry of Tuberculosis. Being a compilation and critical review of existing knowledge on the chemistry of the tubercle bacillus and its products, the chemical changes and processes in the host, the chemical aspects of the treatment of tuberculosis. By H. Gideon Wells, M.D., Ph.D. Director of the Otto S. A. Sprague Memorial Institute, Professor of Pathology in the University of Chicago and in Rush Medical College. Lydia M. DeWitt, M.D., A.M. Member of the Otto S. A. Sprague Memorial Institute, Associate Professor of Pathology in the University of Chicago and in Rush Medical College. Esmond R. Long, Ph.D. Assistant Professor of Pathology in the University of Chicago and in Rush Medical College. Cloth. Price, \$5.00. Pp. 447. Williams and Wilkins Company, Baltimore, 1923.



*Colloids in Biology and Medicine\**

CHEMISTRY in general and physiological chemistry or biochemistry in particular, aims to investigate the structure of individual chemical substances and to explain their properties by splitting and synthesizing them and comparing the rearticulated substances with the original. Colloidal substances, although susceptible to analysis, cannot be entirely resynthesized, and the methods of colloidal research are therefore quite different from those customarily used in organic and physiologic chemistry. Colloidal chemistry consists primarily in the study of the machine as a whole rather than of its component parts. The splitting of proteins into peptones and amino acids is a violent procedure and the study of the resultant split products gives us little thorough information of the nature of the mother substance. The student of biocolloids avoids such profound attacks and strives to keep the molecule intact so far as possible, studying its outward form, the chemical points of attack offered by the unmutilated molecule and its behavior to changes which may occur under normal and pathological conditions.

Colloid chemistry thus becomes a division of chemistry quite apart from the other more familiar methods of study. It approaches more nearly a study of the living molecule.

After a detailed consideration of colloids, colloidal phenomena and methods of colloidal research, the author enters into a discussion of various colloids as individuals, particularly lipoids, proteins and carbohydrates. The major portion of the book is devoted to a practical discussion of colloidal phenomena as they occur in the living body. These studies are grouped under the headings Enzyme Action, Immunity Reactions, Metabolism of the Various Tissues and Systems of the Body, Growth, Metamorphosis and Development, Secretion and Excretion. The chapters on the movements of organisms, blood respiration and circulation are of particular interest. The colloidal properties of foods and condiments and colloidal phenomena as they occur in toxicology and pharmacology are discussed.

As usual, when one becomes intensely interested in a single subject, the author observes the majority of phenomena in terms of colloidal chemistry.

On the whole, the volume may be characterized as reasonably conservative, thoroughly comprehensive, and as one, a study of which offers the solution to many problems in medicine which have not been satisfactorily answered in the phraseology of physiological chemistry.

---

\*Colloids in Biology and Medicine. By Prof. H. Bechhold, Member of the Royal Institute for Exp. Therapeutics in Frankfurt A. M. Authorized Translation from the second German edition with notes and emendations by Jesse G. M. Bullowa, A.B.M.D., Assistant Clinical Professor of Medicine, Fordham University; Adjunct Professor of Clinical Medicine, N. Y. Polyclinic School and Hospital; Visiting Physician Riverside Sanatorium; Associate Visiting Physician Willard Parker Hospital, N. Y. City. Price \$5.00. Pp. 464. 54 illustrations. D. Van Nostrand Co., New York, 1919.



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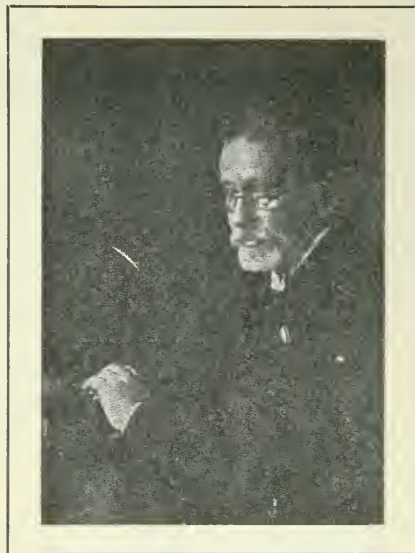
## CHAPTER I.

(For Introduction see June and July Nos.)

### METHYL ALCOHOL IN ARSPHENAMINE (SALVARSAN).

After Ehrlich had finished his salvarsan and began testing its purity, biologically and chemically, he found that the dried salvarsan contained something that was not the drug, or its essence, because the arsenic content of the preparation was too low.

He found salvarsan contained 31.6% arsenic instead of 34.2%, its right content of arsenic. Ordinarily this difference between the actual and the theoretical amount of constituents is due to water of crystallization or impurities or both. In the preparation of salvarsan absolute methyl alcohol was the last solvent used.



Paul Ehrlich, M.D., Pioneer

## WHAT IS THE CONCOMITANT OR IMPURITY THAT MAKES THE ARSENIC CONTENT OF SALVARSAN TOO LOW? IT IS METHYL ALCOHOL.

THOSE who represented it as Water of crystallization or hid its methyl alcohol content:

- (1) Original German Manufacturers.
- (2) British and Canadian Manufacturers.
- (3) American Manufacturers, except one.
- (4) C. N. Meyers and A. G. DuMez, Public Health Reports, No. 25, page 1004, 1918.

THOSE who actually found METHYL ALCOHOL, or who identified and estimated it in SALVARSAN and published it:

- (1) Kober, Journal of American Chemical Soc., vol. xli, 442, (1919).
- (2) Rieger, Journal of Laboratory and Clinical Medicine, vol. iv, 181, (1919).
- (3) Raiziss, Garvon and Falkov, (Dermatological Research Laboratories), Science vol. iv, 23-24, 1922.
- (4) Binz, Bauer and Hallstein, (From Georg Speyer-Haus, Frankfort a. M., The original Ehrlich Laboratory) Berichte der Deutschen Chem. Gesellschaft, vol. lii, 422, (1920).

**SUMMARY.** Therefore Methyl Alcohol is a constituent of the original salvarsan and of arspenamine made according to the original method; in other words, those in use heretofore.

**DANGER.** There is great danger that this methyl alcohol will form methyl derivatives with salvarsan and that these as Ehrlich's Laboratory has shown are toxic and therapeutically inefficient. (Berichte der Deut. Chem. Gesellschaft, xlv, 2135, 1912.)

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Bromphenol Blue	yellow-blue	3.0-4.6
Resorcin Blue (Lacmoid)	pink-blue	4.0-7.2
Methyl Red	red-yellow	4.4-6.0
Bromcresol Purple	yellow-purple	5.2-6.8
Bromthymol Blue	yellow-blue	6.0-7.6
Phenol Red	yellow-red	6.8-8.4
Cresol Red	yellow-red	7.2-8.8
Thymol Blue (Alkaline range)	yellow-blue	8.0-9.6
Cresol Phthalein	colorless-red	8.2-9.8
Phenol Phthalein	colorless-red	8.4-9.2

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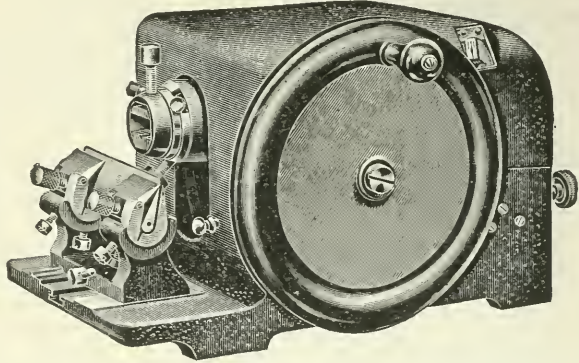
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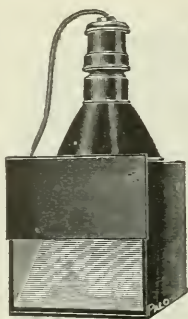
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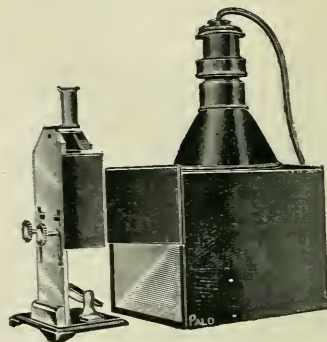


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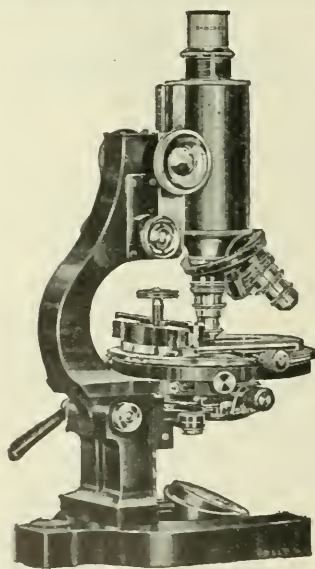
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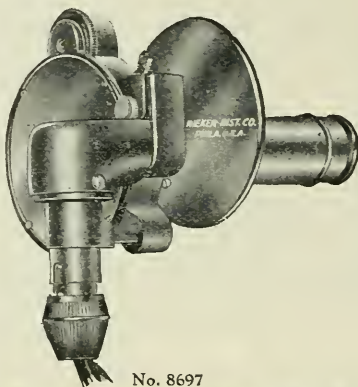


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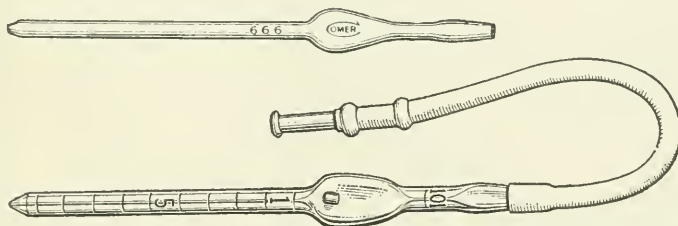
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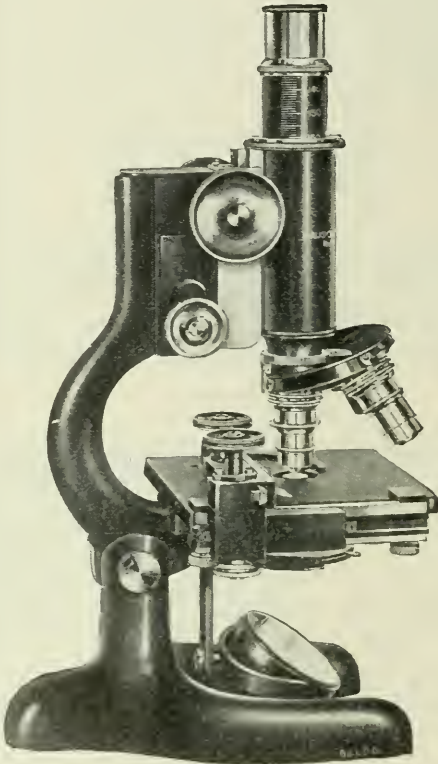
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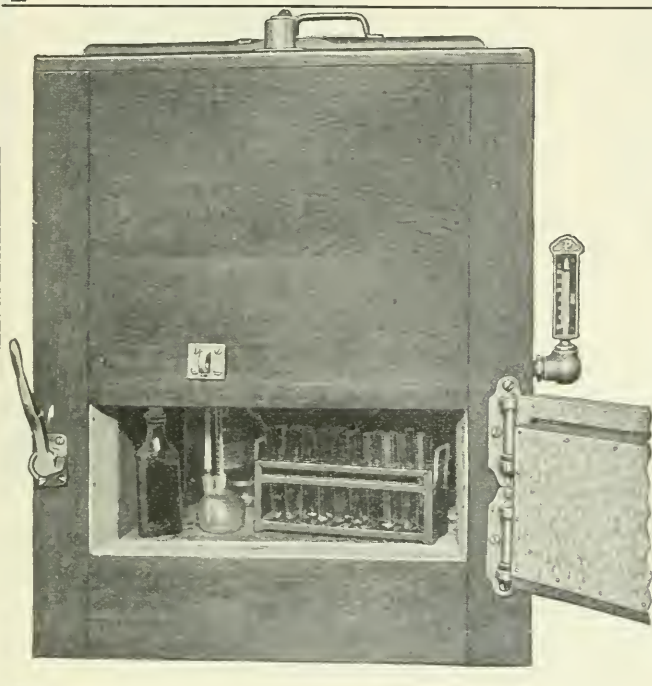
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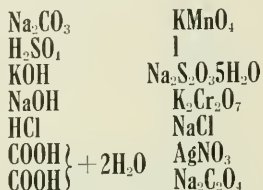
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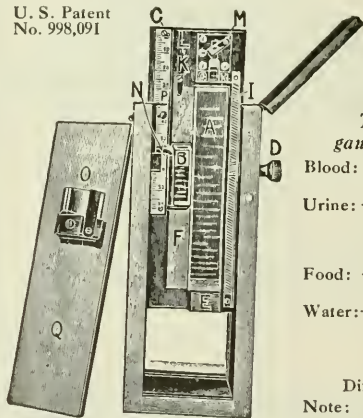
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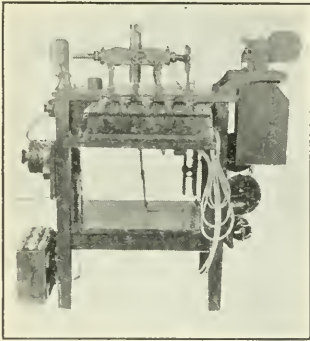
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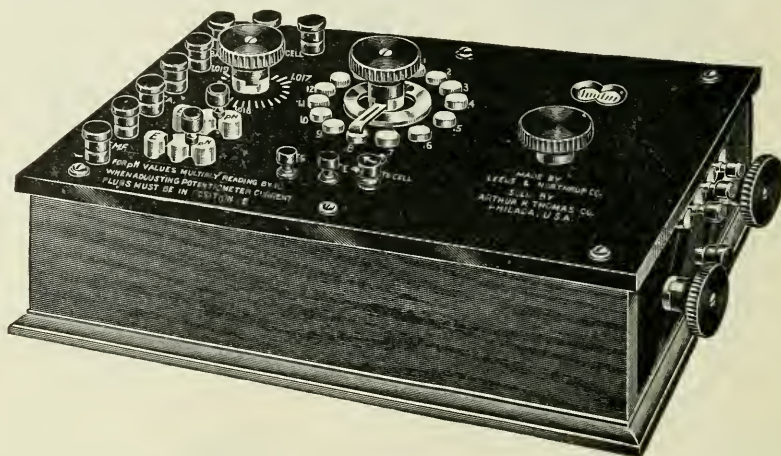
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VOL. VIII

ST. LOUIS, MO., SEPTEMBER, 1923

No. 12

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## ORIGINAL ARTICLES

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### BASAL METABOLIC RATES IN SOME OF THE PSYCHONEUROSES\*

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BY B. S. LEVINE, PH.D.†

---

ONE hundred cases which were referred to the laboratory for basal metabolic rate determinations were classified and studied. Each case exhibited two or more of the cardinal signs of hyperthyroidism, described by Moebius and by Chareot, and the degree of manifestation of the symptoms was well pronounced. Since the patients were sent to this hospital for admitted reasons of nervousness, the basal metabolic rate determinations were resorted to in the attempt to determine, if possible, whether the primary affliction was of a purely nervous or of an hyperthyroid nature.

It cannot be stated with any degree of finality, however, even in the evidence of the most reliable basal metabolic determinations, whether the cause of the symptoms is central, autonomic, or whether it is of an endocrine origin. Even a hasty review of the literature on basal metabolism in its application to the diagnosis of or differentiation between goiter affection and certain, so-called, functional nervous manifestations, exposes a tangle of views, which at first seems almost hopeless. Thus, Means and Aub<sup>1</sup> conclude that basal metabolic determinations differentiate between true thyroidism and simple neurasthenia, and make possible early diagnosis of exophthalmic goiter.

Boothby<sup>2</sup> categorically states that "for practical diagnosis, an increased basal metabolic rate indicates hyperthyroidism, either from exophthalmic goiter or thyroid adenoma, provided active acromegaly is ruled out and the group of febrile diseases is eliminated by a normal temperature curve." He states further that "the basal metabolic rate is of the utmost value in the differential diagnosis of mild hyperthyroidism (of either type) and a neurosis

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\*Approved for publication by the Surgeon General, U. S. P. H. S.

†Director of Clinical Laboratory U. S. Veterans' Hospital, No. 37, (N-P), Waukesha, Wisconsin.

simulating hyperthyroidism which often may be associated with an enlarged colloid goiter or a thyroid adenoma which is not producing an excess of the thyroid hormone \* \* \* An increased metabolic rate in a neurotic patient—he believes—is due to the fact that the rate was not obtained with the patient under basal or standard conditions.”

In another paper the same author<sup>3</sup> states that “in the differential diagnosis of adenoma with hyperthyroidism and exophthalmic goiter it is obviously necessary to exclude definitely cases presenting the syndrome of neurasthenia, usually of the cardiac type, with many of the earmarks of hyperthyroidism, but in which no hyperthyroidism or overactivity of the thyroid is usually present.” In further emphasizing his belief in the sufficiency of the basal metabolic rate determination in establishing or substantiating a diagnosis, this author states that “patients having simply neurasthenia, even those whose symptoms most closely simulate hyperthyroidism, do not have persistently elevated basal metabolic rates.” In this article he again states emphatically that in his opinion “a normal basal metabolic rate eliminates hyperthyroidism.” In another article<sup>4</sup> the same author again states that “basal metabolic rate serves as an accurate diagnostic aid in the recognition of the presence or absence of hyperthyroidism.” Sandiford<sup>5</sup> believes as does Boothby, and states that “the basal metabolic rate is of greatest value in thyroid disorders because it gives a *very accurate mathematical index* (the italics are the writer’s) of the degree of functional activity of the thyroid gland. \* \* \* No definite instance of an increased basal metabolic rate has been found in that group of cases known as neurasthenia or chronic nervous exhaustion. The basal metabolic rate has proved, therefore, to be of great value in the differential diagnosis of neurosis simulating hyperthyroidism, and true hyperthyroidism.” McGuire<sup>6</sup> believes that a patient with hyperthyroidism may have a high metabolic rate and not be as seriously ill as another having a lower rate but whose structural changes in the heart, kidney, and liver are rather pronounced. This is in seeming contradiction with the above cited opinion of Sandiford. He believes, however, that the onset of hyperthyroidism is insidious and difficult to recognize in its early stages, when the metabolic rate clearly differentiates it from hysteria, neurasthenia, and other conditions with which it may be confused.

Woodbury<sup>7</sup> comes to the conclusion that thyrotoxic psychosis does not exist as a distinct entity, but that commonly designated neurotic or psychotic symptoms arise in thyrotoxic cases. These can be separated from psychoneurotic states free from endocrine involvements by determining their basal metabolic rate. Miller and Raulston<sup>8</sup> frequently observed patients in whom the diagnosis of neurasthenia has been made, but in whom manifestations similar to those observed in hyperthyroidism were present. In the light of Cannon’s experimental observations, they conclude that in this type of patients there is continually present in the blood an increased thyroid secretion, and that on excitement or moderate exertion this secretion further increases to an extent sufficient to bring forth definite symptoms of hyperthyroidism. Therefore, for purposes of study, they feel justified in consider-



ing as a possible hyperthyroidism every case in which the chief complaint is nervousness.

It is believed by those holding the above cited views that the degree of the so-called hyperthyroidism and, hence, the height of the metabolic rate accompanying it, is proportional to the degree of the secretory activity of the thyroid gland. In this connection Kessel and his collaborators<sup>9</sup> make the following statements: "The potency of the thyroid hormone is proportional to its iodine content. In the hyperplastic gland of the exophthalmic goiter there is a diminution of iodine. There is no direct proof that an increased thyroid secretion is present in hyperplasia. What data are available indicate a decreased output and storage." And as for elevation of basal metabolism, they believe that it may occur in other conditions dissociated from goiter or alterations in the involuntary nervous system. In another paper the same authors,<sup>10</sup> after a study of 86 cases possessing the classic symptomatology of exophthalmic goiter, come to the conclusive opinion that it is unwise to designate the condition of exophthalmic goiter as an endocrine disorder, and they, therefore, propose the abolition of such terms as "hyperthyroidism" and "dysthyroidism." Their experience had impressed them with the fact that the majority of patients presenting exophthalmic goiter give a history past or present of autonomic imbalance. Since the causative relation between autonomic imbalance and exophthalmic goiter has not been established, they do not commit themselves, but think it improbable for thyroid hyperplasia to be the cause of the imbalance.

Brooks<sup>11</sup> likewise believes that preexisting emotional states are the primary causes of the conditions frequently designated as Graves' disease. If the condition is a transient one, he calls it, "Physiological hyperthyroidism" and thinks it "is brought about by an abnormally great demand on the thyroid as the result of developmental growth associated with too much emotional output. When these factors are either diminished within the normal limits or directed into other channels, automatically the overaction of the gland, together with other secondary factors that may be associated with it, drop toward the normal; but if the urge is too long continued, or if fixed habits of demand and response be established, the condition will develop either into Graves' disease or take on a neurasthenic phase likely to ruin the life of the individual. It appears, then, to be but a normal response to an exaggerated or abnormal demand with the final establishment of a tissue habit." These statements of Brooks are based upon long continued and careful observation, and although lacking the backing and convincing reasoning of carefully planned experimentation, embody in them views which may be considered as strong probabilities.

More perplexing to the clinician, who seeks for an unequivocal significance in the basal metabolic values, is the view of Carlson<sup>12</sup> who speaks of "apparent reliable clinical reports to the effect that the, so-called, hyperthyroidism or toxic goiter may be present with a normal metabolic rate and that hyperthyroidism or myxedema may be present and parallel with a high or normal basal metabolism. \* \* \* Mere increase in the size of the gland,



mere hyperplasia does not mean more gland activity or more secretion. Thyroid hyperplasia and thyroid adenoma occur without physiologic disturbances, although the size, structure and the chemistry of the thyroid seem to be identical with those in the toxic goiter." He, too, believes that the primary relation of exophthalmic goiter to the thyroid gland has not been definitely established.

An interesting and instructive contradiction is afforded by the following two papers in which "hysteria" appears to be the central factor. Juarros<sup>13</sup> speaks of the frequency with which diagnosis confuses hysteria and hyperthyroidism in women. He thinks this is due to an equivocal conception of hysteria. To avoid such confusion he looks for symptoms of hyperthyroidism in all women with manifestations simulating hysteria, but in whom the so-called hysterical mentality (?) is lacking. (The interrogation mark is the writer's.) In other words, he believes that *the hysterical manifestations are caused by the existing hyperthyroidism*. How erroneous this may be is shown by an experience of Danna<sup>14</sup> who reports a case of a boy with a so-called well defined *psychoneurosis, hysterical type*, which was entirely relieved of the neurotic symptoms *through the administration of thyroid treatment*. Aub, who as previously cited, believed in 1919 the basal metabolic rate determinations to be a safe means for the recognition of exophthalmic goiter, later<sup>15</sup> expressed the opinion that the regulation of metabolism is also under the control of many factors independent of the internal glands, and, hence, of the thyroid.

Timme<sup>16</sup> has well summarized the situation, and the writer takes the liberty of citing considerable of his statement. He says: "The endocrine system has no constant quantities, only variables which need a higher type of mathematics, a calculus which furnishes information about differentials, but the end result has to be integrated by integral calculus. \* \* \* The answer to any integral calculus problem dealing with variables is never one term or absolute quantity. It is always represented in a series of terms combined in a formula of functions of the factors involved. To illustrate: A middle-aged woman gives the clinical aspects of myxedema: her basal metabolic rate was determined by three investigations made by two investigators and varied from +80.0 per cent to +100.0 per cent. Hence, the man who saw her refused to believe it was myxedema, and refused to give her thyroid. Examining the case further, we found that she had intense headache, and roentgenographic examination showed she had an enlarged sella turcica and a bitemporal hemianopsia with a diminution in vision. Therefore, it was a case of hypophysial mass, plus myxedema.

She was given thyroid. Within two or three days she began to improve; her headaches began to diminish, and the basal metabolic rate became lower, on no other treatment than the administration of the thyroid. It does not mean that the deficiency of the thyroid produced a high metabolic rate; the high rate may be due to the pressure on parts of the brain. In any event, it means that the integration of the syndrome is a formula, and that is what we have to consider in all the innumerable problems which con-

front us, and the formula is one which involves all the varying activities of the glandular elements."

Regardless as to whether the study of the metabolic values of these cases will point towards an increased, normal, or low rate in any of the ordinarily designated nervous afflictions here classified, in the light of the above review of the opinions on the subjects of hyperthyroidism, neuroses, and metabolism, we feel justified in saying, *a priori*, that it will add nothing definite to the determination of the causes of these afflictions. Nervous manifestations forming the syndromes according to which the cases studied in this paper have been classified, like metabolism, are mere symptoms, and, by all probability, are not called forth by the same cause or combination of causes. We come to realize more and more that in the realms of the psychoses and psychoneuroses as much confusion prevails at present as in the general field of endocrinology. Hence, in the following study we aim to point out the extent of increased or decreased basal metabolism and the frequency of their occurrence in the neuroses groups under investigation.

The method used in the determination of the metabolic rate was the same as was described by the writer<sup>17</sup> in a previous publication. It may be appropriate to remark at this point that emotional states, even when sufficiently concealed or suppressed, have a great effect upon the metabolic rate of all subjects, and especially of those afflicted with psychoneurotic instabilities. Such patients may be at perfect physical rest during the test period, and yet yield a metabolic rate of from +10.0 per cent to +50.0 per cent above their true basal metabolic rate. Occasionally an emotional state will result in a reduced rate, but this happens very seldom. The factors causing such an emotional state may be: a fleeting stimulating thought of a pleasant or unpleasant nature; a sexual stimulation due to the presence of a person who directly or through association stimulates the patient; the personality of the technician; the fear of the patient that the test will disclose to the physician that which he is carefully trying to conceal; and many others.

Such increase in metabolism, however, is not due to the thyroid secretion, since the effects of the stimulation are registered almost instantaneously. It is well established now that the response of metabolism to thyroid secretion is considerably delayed, and is quite gradual as regards both, the increase and decrease in its intensity, as was shown by Plummer and Boothby.<sup>18</sup> On the other hand, an increase in the secretion of the suprarenal glands causes a rapid and marked increase in the metabolic rate, which declines almost as rapidly. Boothby and Sandiford<sup>19</sup> showed that adrenalin not only caused an invariable increase in the heat production of their test subjects, but that it also occasioned an increase in the ventilation and in the respiratory quotient. We found in our neurotic patients, who showed a higher metabolic rate than their true basal, on a repeat test, that there was a change in the rate of their ventilation and in the respiratory quotient, and we conclude from this that the high metabolic rate was due not to the thyroid overaction, but to the stimulation of the suprarenals.

In any event, the above discussion indicates, from a technical point of

view, that in determining basal metabolic rates on neurotic patients the following points must be carefully observed: (1) that it be done by a competent person who understands neurotic patients and who is more or less familiar with their individual histories and who knows how to gain their confidence, (2) that more than one test be made previous to forming final judgment and (3) that an apparatus which reliably registers the ventilation rate and the respiratory quotient be used in preference to any other make.

For purposes of uniformity and for more universal understanding of the results, the rates obtained in our study were recorded in terms of plus and minus percentile variation from a known "standard of comparison." This standard was 39.5 calories per square meter of body surface area per hour, and is the Aub and DuBois standard of normal rate for males between the ages of 20 and 40 years. It was used in this study as the "standard of comparison" because none of our patients exceeded the age of 40.

The expression "standard of comparison" is here used in preference to the expression "normal standard," as an indication of the probability of the adopted "normal standard" not being sufficiently reliable. In a personal conversation with the writer, Dr. W. M. Boothby has expressed the opinion that the DuBois standard may not be regarded as a finality in its adequacy and should be supplemented by additional determinations in the thousands, if possible. Even then, the question as to whether a "standard" so selected represents a "normal" condition remains an open one. It is possible that the case in this instance is similar to the case of the "normal gastric acidity." It has been shown by Rhexuss, Hawk, and their collaborators<sup>20</sup> that there are normally high gastric acidities. This was corroborated by the writer<sup>21</sup> who pointed out that there are also normally low gastric acidities and that the degrees of the acidities are not to be regarded as the factors of importance, since they may vary considerably in the same individual, but that the constancy of the so-called "percentile relationship of the titrable factors" may prove to be the determining factor of normality. Something similar may be true with regard to basal metabolic rates. Indeed, in another paper we shall show that this view has a high degree of probability.

In the numerical order, the cases here reported were as follows: Hysteria, 42; Neurasthenia, 25; Anxiety, 16; Organic, 8; Constitutional psychopathic states, 4; Traumatic neurosis, 3; Alcoholics, 2. Summaries of the rates of each of the groups are given in the corresponding tables following.

As seen from Table I. 42 cases with a primary diagnosis of hysteria were tested with a total of 96 basal metabolic determinations. Of the group as a whole, three determinations did not vary from the standard of comparison, and were, therefore, denoted as  $\pm 0.0$  per cent; thirty-two determinations had a percentile variation between  $\pm 0.0$  per cent and  $+5.0$  per cent ten determinations had a percentile variation between  $+5.0$  per cent and  $+10.0$  per cent; the rate of fifteen determinations exceeded  $+10.0$  per cent. Seventeen determinations had a variation between  $\pm 0.0$  per cent and  $-5.0$  per cent; twelve determinations possessed a variation between  $-5.0$  per cent and

-10.0 per cent; eleven determinations were below -10.0 per cent. In terms of cases, twenty-four, or 57.0 per cent had an average rate variation between  $\pm 0.0$  per cent and +10.0 per cent; nine cases, or 21.0 per cent possessed a rate variation between  $\pm 0.0$  per cent and -10.0 per cent, making a total of 78.0 per cent of cases of this group possessing a rate lying within the  $\pm 10.0$  per cent limits.

TABLE I—THE GROUP OF HYSTERIAS

NO.	NAME	BASAL	METABOLIC	RATES	EXPRESSED	IN	PERCENTILE	VARIATION
1	N. J. H.	+ 5.0%	+ 2.5%					
2	R. C.	- 2.5	- 0.2					
3	G. S.	+11.1	- 1.0	+ 1.5				
4	C. M.	- 1.5	- 6.6					
5	V. A. B.	+ 2.0	- 6.0	$\pm 0.0$	- 9.3			
6	F. B.	- 9.6	- 8.6	-27.3	-30.0			
7	W. B.	+11.6	+14.6					
8	W. DeC.	- 5.0	- 5.0					
9	M. O. C.	+ 3.6	+ 7.3					
10	A. R. C.	- 9.6	- 9.6	- 6.3	-13.0			
11	H. G. D.	+ 1.8	+ 1.8					
12	R. J. D.	+ 1.7	+ 0.5					
13	F. A. D.	+ 4.0	+ 7.0					
14	H. J. D.	- 1.5	- 2.3					
15	E. B.	+ 4.8	+ 1.3					
16	W. H.	+ 3.8	+ 3.8	+ 3.8				
17	F. H.	+ 5.0	+ 4.6					
18	H. J.	+ 3.5	+ 3.5	- 1.3	- 3.5			
19	R. J.	+ 8.6	+10.8					
20	H. T. J.	+ 1.3	+ 1.5					
21	X. K.	- 0.3	- 3.0					
22	A. L.	- 1.5	+ 3.5					
23	P. O. M.	-26.3	-24.8					
24	E. M.	+15.2	+15.7					
25	X. R.	- 5.0						
26	Ras.	+ 5.5	+ 4.3					
27	H. R.	+14.0	+14.0					
28	H. S.	+ 8.0	+10.9					
29	Spr.	+11.4	+13.2					
30	Gos.	- 6.5	- 3.1	- 6.3	- 8.0			
31	E. W. T.	+ 2.3						
32	A. S.	+ 1.2	- 0.9					
33	W. R.	+ 9.2						
34	J. E.	+12.3	+11.3	+13.0	+13.4			
35	G. A.	+ 8.9	$\pm 0.0$					
36	H. S.	+ 5.5						
37	Mu.	+32.9	+36.7					
38	M. O.	$\pm 0.0$	- 0.5	+ 6.8				
39	L. R. B.	+ 3.5	+ 1.0					
40	T. B.	+ 0.1	+ 0.8					
41	D. D.	-11.0	-50.0	- 7.3				
42	W. W.	+ 8.8	+ 1.5					

Six cases, or 15.0 per cent possessed a rate variation exceeding +10.0 per cent, and three cases, or 7.0 per cent possessed rates with a variation below -10.0 per cent, making a total of 22.0 per cent of cases of this group which, according to the prevailing standard, would be regarded as out of the normal range. Considering that all the cases of this group showed a number of the cardinal signs of disturbed metabolism, and that they were sent to the laboratory as suspicious cases, the percentage of those having



rates which might be regarded as either excessive or insufficient is surprisingly low.

Classifying the cases of this group into subgroups, according to the amends in the diagnoses, it was found that nineteen cases were diagnosed as pure hysteria; of these eleven, or 60.0 per cent possessed rates not exceeding +10.0 per cent; five cases, or 26.0 per cent possessed rates lying between  $\pm 0.0$  per cent and -10.0 per cent, making a total of 86.0 per cent of cases exhibiting metabolic rates which are not regarded at present as either too high or too low. Three cases, or 14.0 per cent possessed rates exceeding +10.0 per cent, and none fell below -10.0 per cent. Hence, the percentages of the average, high, and low rates in this subgroup are practically the same as the percentages of the group as a whole.

The next subgroup was found to consist of six cases in which endocrine involvements, chiefly of the thyroid type, have been definitely added to the primary diagnosis of hysteria. The vagueness of the statements of the secondary diagnoses in these cases and the metabolic rates are worthy of note. They were as follows:

1. Hysteria with hyperthyroidism, with metabolic rates of -1.5 per cent, and -2.3 per cent.
2. Hysteria with toxic goiter, with metabolic rates of -0.3 per cent, and -3.0 per cent.
3. Hysteria with endocrine imbalance, with metabolic rates of +8.0 per cent; and +10.9 per cent.
4. Hysteria with dysthyreosis, with a metabolic rate of +2.3 per cent.
5. Hysteria with endocrinopathy, with a metabolic rate of +9.2 per cent.
6. Hysteria with endocrine imbalance, with metabolic rates of +8.9 per cent and  $\pm 0.0$  per cent.

It is perhaps of significance that none of these cases possessed rates above +10.0 per cent, whereas in the pure hysteria cases occurred with rates as high as -35.0 per cent. However, the average values of this subgroup, each case of which exhibited the cardinal signs of Basedows' disease to such an extent that the gland involvement was definitely indicated in the diagnosis, were practically the same as the averages of the subgroup of simple hysterias. Thus, the average of the plus values of the simple hysterias was +6.65 per cent, and of the hysterias with suspected endocrine disturbance +6.7 per cent. The average of the minus values of the pure hysterias was -3.8 per cent, and of the hysterias with the endocrine status under suspicion was -1.8 per cent. The total average of the pure hysterias was +3.7 per cent, and that of the hysterias with the endocrine status under definite suspicion was +3.2 per cent.

Of the subgroup of five cases diagnosed as hysteria with the admixture of anxiety expressions, two cases possessed rates exceeding +10.0 per cent, the rate of one was below -10.0 per cent, the rate of the two others were within the  $\pm 10.0$  per cent limits. It may seem from these data as though the presence of the disturbance which results in manifestations conveniently designated as anxiety, is paralleled by higher metabolic rates, but as will be

shown under the discussion of the group of anxiety cases, this is only seemingly so, and is probably due to the small number of cases under consideration.

Three cases were diagnosed as hysteria with psychopathic manifestations, all of which possessed basal metabolic rates not exceeding +10.0 per cent. Two cases were diagnosed as hysteria on the basis of mental deficiency, one of which did not exceed +10.0 per cent, while the other had a rate considerably below -10.0 per cent. Two cases were diagnosed as hysteria with hypochondriacal tendencies, the rates of both were within the  $\pm 10.0$  per cent limits. Two cases were diagnosed as hysteria with bradycardia; of these one had an average rate of -16.4 per cent, the other had an average rate of +12.3 per cent. One case was diagnosed as hysteria with neurasthenic admixtures, one as hysteria with alcoholic tendencies, and one as hysteria on the basis of hermaphroditism. The first possessed rates not exceeding +10.0 per cent, the average rate of the last one was of a minus sign, but not below -10.0 per cent. It may be seen from this outline that of all the subgroups discussed, the one of the simple hysterias indicates a more frequent occurrence of cases with rates lying out of the  $\pm 10.0$  per cent limits, and that the coexistence, with the factors causing hysteria, of other disturbances leading to the apparent necessity of so-called secondary diagnoses, has no effect upon the basal metabolic rates.

#### THE NEURASTHENIAS

Twenty-five cases were diagnosed as neurasthenias, and their rates were as shown in Table II.

As may be seen from Table II, the total of the rate determinations was 64. Of the group as a whole, two determinations equaled  $\pm 0.0$  per cent; ten determinations had a percentile variation not exceeding +5.0 per cent; thirteen had a percentile variation not over +10.0 per cent and thirteen exceeded +10.0 per cent. Five determinations had a percentile variation between  $\pm 0.0$  per cent and -5.0 per cent; fourteen between -5.0 per cent and -10.0 per cent, and two were below -10.0 per cent. In terms of cases, 10, or 40.0 per cent had an average percentile variation not exceeding +10.0 per cent; eight cases, or 32.0 per cent had percentile variations not lower than -10.0 per cent; making a total of 72.0 per cent of cases of this group possessing rates which are considered according to the present standards as neither too high nor too low. Six cases, or 24.0 per cent, possessed rates with a percentile variation exceeding +10.0 per cent, and one case, or 4.0 per cent, possessed a rate with a percentile variation below -10.0 per cent, thus making a total of 28.0 per cent of the cases of this group which according to the present adopted standard would be regarded as out of the normal range.

Comparing these percentages with those of the group of the hysterias, it is seen that they are almost identical in their totalities, (78.0 per cent as compared with 72.0 per cent of cases with rates within the  $\pm 10.0$  per cent limits, and 22.0 per cent as compared with 28.0 per cent of cases without the  $\pm 10.0$  per cent limits) and considering that in this group, as in the group of hysterias, the cases showed pronouncedly the cardinal signs of disturbed

metabolism and that some of them were previously established as "high raters," the percentages may be regarded likewise as surprisingly low. Since neurasthenias more generally than hysterics have been confused with cases of thyroid or metabolic disorders, in the plus direction, it might be supposed that the average rates of the neurasthenics, especially of those lying without the adopted high normal limit would be greater than those of the hysterics. This, however, does not prove to be the case. Thus, the average percentile rate variation of the hysterics whose rates exceeded +10.0 per cent was 16.4 per cent, and that of the corresponding cases among the neurasthenics was 17.7 per cent.

TABLE II

NO.	NAME	BASAL	METABOLIC	RATES	EXPRESSED IN PERCENTILE VARIATION
43	McM.	- 4.1%	- 2.9%	- 8.3	
44	R. L. S.	- 4.8	- 6.0		
45	W. S.	- 6.1	- 6.0		
46	G. S.	- 7.3	- 7.6		
47	V.	- 5.6	- 3.5		
49	W. T. H.	+ 5.8	+ 3.0		
48	B.	+24.0			
50	R. C.	- 5.4	± 0.0		
51	C. D.	+ 5.0	+11.7	+12.6	+14.4
52	G. R. F.	+ 0.3	+ 3.4	+ 3.4	- 3.0
53	G. B. G.	- 6.6	-12.6	-11.4	
54	S.	+ 6.9	+ 6.9	+ 6.1	- 3.86
55	A. F.	+ 9.6	+ 8.0		
56	Th. M.	+ 9.0	+11.1	+12.7	
57	L. F.	+ 3.6	+ 6.6		
58	B. C.	+ 8.0	+ 6.6		
59	W. C.	+16.5			
60	F. W.	+17.5	+17.5	+18.5	+18.5
61	H. H. R.	+ 8.6	+ 7.8	+ 8.6	
62	T. D. C.	+ 2.5	± 0.0		
63	J. N.	- 1.0	+ 5.5		
64	D. R.	+40.5	+37.0		
65	C. R.	- 7.1	- 4.8	+ 2.3	+ 5.1
66	F. L.	+ 6.6	+ 3.6		
67	A. C.	- 8.5	- 8.5	- 6.7	- 7.0

Classifying the cases of this group into subgroups according to their amended diagnoses, it was found that six cases were diagnosed as pure or simple neurasthenias. Of these, four cases, or 80.0 per cent, possessed basal metabolic rates with percentile variations between -3.0 per cent and -9.0 per cent, one case possessed a basal average rate of +4.4 per cent and none were without the limits of +10.0 per cent. The average of the plus values of this group was +4.4 per cent, that of the minus values -5.62 per cent, and the total average was -3.95 per cent. These figures are rather interesting as compared with the figures of the corresponding group of the hysterics and point to a lower metabolic rate among the so-called simple neurasthenias than among the simple hysterics.

Eight cases were diagnosed as neurasthenia with endocrine involvements of the thyroid type. They were as follows:

1. Neurasthenia with thyroidectomy residuals, with B. M. Rates +24.0 per cent.

2. Neurasthenia with mild dysthyreosis, with B. M. Rates  $-5.4$  per cent, and  $\pm 0.0$  per cent.

3. Neurasthenia with mild hyperthyroidism, with B. M. Rates  $+5.0$  per cent,  $+11.0$  per cent,  $+12.6$  per cent,  $+14.4$  per cent.

4. Neurasthenia with pronounced tachycardia and with hyperthyroidism strongly suspected, with B. M. Rates  $+0.3$  per cent,  $+3.4$  per cent,  $+3.4$  per cent,  $-3.0$  per cent.

5. Neurasthenia with hysterical admixtures and with endocrine disturbance, with B. M. Rates  $-6.6$  per cent,  $-12.6$  per cent,  $-11.4$  per cent.

6. Neurasthenia secondary to hyperthyroidism, with B. M. Rates  $+6.9$  per cent,  $+6.9$  per cent,  $+6.1$  per cent,  $-3.8$  per cent.

7. Neurasthenia with hyperthyroidism under suspicion, with B. M. Rates of  $+9.6$  per cent,  $+8.0$  per cent.

8. Neurasthenia with probable dysthyreosis, with B. M. Rates  $+9.0$  per cent,  $+11.1$  per cent,  $+12.7$  per cent.

It is thus seen that three cases, or  $37.5$  per cent of this group had a metabolic percentile variation exceeding  $+10.0$  per cent, whereas none of the cases of the corresponding subgroup of the hysterics exceeded this rate. This points to the probability of "high metabolic raters" occurring more frequently among neurasthenics suspected of having metabolic disturbances, than among the corresponding hysterics, although as stated above, the average metabolic rates of the groups are nearly the same. The one case possessing a metabolic rate with a percentile variation below  $-10.0$  per cent was a special case and will be discussed elsewhere.

Two cases were diagnosed as neurasthenia with psychasthenic admixtures, one of which also had marked homosexual tendencies. The latter will likewise be discussed in greater detail in a following paper. Their metabolic rates were for the first  $+16.5$  per cent, and for the second  $+17.5$  per cent,  $+17.5$  per cent,  $+18.5$  per cent, and  $+18.5$  per cent. One case was diagnosed as neurasthenia with gastrointestinal toxicosis and had rates of  $+40.0$  per cent, and  $+37.0$  per cent. The remaining cases were all within the  $\pm 10.0$  per cent limits, indicating that such concomitants as mental subnormality, renal dysfunction, tuberculosis, progressive muscular atrophy, have no effect upon the basal metabolic rate of the cases studied in this group, provided their temperature curve is normal.

#### THE GROUP OF ANXIETIES

There were sixteen cases with a primary diagnosis of anxiety, as shown in Table III.

Eight of the cases were diagnosed as pure anxiety, the rest having concomitants or admixtures such as mitral regurgitation, chronic nephritis, hysterical and psychasthenic manifestations, mild myocarditis, etc. One of the sixteen cases, diagnosed as pure anxiety, had a basal metabolic rate of  $+16.5$  per cent and  $+15.7$  per cent, and one, with valvular heart disease, possessed metabolic rates of  $+13.0$  per cent and  $+13.8$  per cent, making a total of  $12.5$  per cent of cases of this group with metabolic rates exceeding the  $+10.0$



per cent limit. This is 2.5 per cent less than the percentage of the corresponding rates among the hysterics and 11.5 per cent less than the percentage of the same among the neurasthenics. The rates, too, are nearer the so-called border line.

The rates of the remaining cases of this group were within the  $\pm 10.0$  per cent limits, indicating, as did the previous two groups, that admixtures or concomitants have no effect upon the rate of the basal metabolism of persons exhibiting the so-called anxiety syndrome.

TABLE III

NO.	NAME	BASAL	METABOLIC	RATES	EXPRESSED IN PERCENTILE VARIATION
68	F. G. C.	- 4.0	$\pm$ 0.0	+ 2.3	+6.3
69	Hud.	- 0.8			
70	Sen.	- 2.8	+ 0.8		
71	M. H. L.	- 3.5	- 7.0		
72	W. K.	- 5.3	- 6.3	+ 5.3	+ 4.8
73	A. K.	- 4.0	- 2.8		
74	F. A. G.	+10.1	+ 9.1		
75	M. E.	- 2.0	+ 2.3	+ 9.8	+ 8.0
76	H. E.	+ 7.9	+ 5.6		
77	McC.	+16.5	+15.7		
78	E. E. A.	- 2.2	+ 1.5		
79	P. C.	+ 7.1	+ 4.6		
80	F. C.	+ 9.6			
81	A. F.	+ 5.0	+ 8.0		
82	Swn.	- 0.5	- 0.7	- 3.5	- 6.1
83	F. S.	+13.0	+13.8		

The group of the constitutional psychopathic states consisted of four patients, all of the type commonly designated as inadequate personality. They were as follows:

- No. 84. N. L. with a basal metabolic rate of +20.5%
- No. 85. F. E. with a basal metabolic rate of + 1.5% and +5.5%
- No. 86. C. H. C. with a basal metabolic rate of + 8.8% and -1.5%
- No. 87. B. B. with a basal metabolic rate of + 6.8% and +8.4%

It is the writer's conviction that the rate of the first case was not basal, and that on repetition it would not exceed +10.0 per cent.

Three cases of traumatic neurosis possessed the following rates:

- No. 88. R. J. C. with a basal metabolic rate of + 4.3%
- No. 89. G. S. with a basal metabolic rate of + 0.5% and  $\pm 0.0\%$
- No. 90. B. C. S. with a basal metabolic rate of + 4.0% and -0.7%

Reference could be made at this point to literature indicating that traumas of various types had definite effects upon the emotional states of the persons affected and causing the appearance of symptoms and even syndromes simulating those commonly recognized at present as due to hyperthyroidism or to exophthalmic goiter, and point out that none of our cases possessed rates of any magnitude deserving attention. However, since the literature reviewed on this subject by the writer dealt with the immediate effects of the traumatic injuries upon the general picture of the patient, and our rates were taken nearly four years after the injuries occurred, the discussion and the comparisons would prove valueless, or questionable in the least.

Two cases of alcoholism had the following rates:

No. 91.	E. C. H.	B. M. Rates	-5.7%
No. 92.	H. V. K.	B. M. Rates	+1.0%

A group of eight organic cases were subjected to basal metabolic rate measurements and are given below:

No. 93.	J. H.	Dysthyreosis, exophthalmic type with B. M. Rates of	-0.5%, -0.9%, -0.5%
No. 94.	H. S.	Exophthalmic goiter, B. M. Rate	-2.5%
No. 95.	B. R. A.	Hyperthyroidism, B. M. Rates	-3.0%, +1.8%
No. 96.	N. E. L.	Tuberculosis, pulmonary, incipient, B. M. Rates	+1.90%, -0.8%
No. 97.	M. H.	Bronchitis, chronic, B. M. Rates	-5.8%, $\pm 0.0\%$
No. 98.	S. B.	Neurosyphilis, B. M. Rates	-8.0%, -6.1%
No. 99.	O. S.	Residuals, encephalitis epidemica, B. M. Rates	+4.0%, +0.7%, -1.5%, -3.0%
No. 100.	L. B.	Exostoses, B. M. Rates	+5.0%, $\pm 1.2\%$

It is worthy to note that the three patients of this group which were diagnosed as primary thyroid cases with practically no outstanding symptoms recognizable by the psychiatrist as indicative of N-P involvements all had rates lying within the  $\pm 10.0$  per cent limits, and that none had an average metabolic rate with a plus sign. The other cases necessitate no discussion.

#### SUMMARY AND DISCUSSION

One hundred of the cases admitted to this hospital during the previous two years were tested for their basal metabolic rates because of the presence of two or more of cardinal signs believed to be indicative of exophthalmic goiter. Only eight of the one hundred cases were found to be affected with primary organic afflictions, the remaining 92 being classified primarily as cases of a neuropsychiatric type. Of the group as a whole 15 cases, or only 15 per cent possessed rates with a percentile variation exceeding the +10.0 per cent limit, and only four cases, or 4.0 per cent possessed rates with a percentile variation below the -10.0 per cent limit, making a total of 19.0 per cent of cases with basal metabolic rates lying without the  $\pm 10.0$  per cent limits. Kay<sup>21</sup> has examined one hundred cases belonging for the most part to the Great Lakes districts which were referred to him for basal metabolic rate determinations for the purpose of substantiating or disproving the temporary diagnosis of hyperthyroidism. He found that only about 33.0 per cent of his cases possessed rates exceeding the +10.0 per cent limit. Among our patients, most of whom likewise belong to the Great Lakes districts, only 15.0 per cent possessed rates exceeding the +10.0 per cent limit.

Further statistical study shows that the average of the plus rates of the group as a whole is +7.4 per cent, that of the minus rates -6.5 per cent, and the total average rate is +2.0 per cent. Less than 30.0 per cent of the cases studied by us had metabolic rates the average of which was qualitatively of a minus type. Less than one-third of these, or only about 10.0 per cent were below the -10.0 per cent limit. Bowman and Grabfield<sup>22</sup> studied fifty mental cases most of whom were insane, some suffered with epilepsy, cretinism, or myxedema. They found that 42 of their cases, or 84.0 per cent possessed basal metabolic rates with a minus sign, and expressed the opinion that the tendency towards a low basal metabolism in cases of mental disease is of

importance and merits consideration in formulating theories as to etiology and treatment. Our findings in connection with cases which may be designated as mild psychoneuroses differ radically from the findings of the above authors, and show that the group types of mental afflictions studied by Bowman and Grabfield and those studied by us affect the metabolic processes of the individuals in different directions.

The percentage of cases with rates exceeding the +10.0 per cent limit among the groups reviewed by us were as follows:

Hysterics	15.0%
Neurasthenics	24.0%
Anxieties	12.5%

indicating a more frequent occurrence of "high raters" among neurasthenics suspected of hyperthyroidism than among any of the other neuroses. The coexistence of secondary psychoneurotic manifestations or of organic concomitants of the types met with in our cases does not seem to influence the basal metabolic rate in any way.

The percentages of cases with metabolic rates below the -10.0 per cent limit were:

Hysterics	7.0%
Neurasthenics	4.0%
Anxieties	None

Our close observation of psychoneurotics with high metabolic rates has led us to the conclusion that the high rates in most instances are not due to an hyperthyroidism or exophthalmic goiter superimposed upon a well defined neurosis, but rather that the hyperthyroidism or the exophthalmic goiter are the direct consequences of or rather the necessary response to the mental state of the patients. Consequently any amount of surgical treatment such as ligation or even excision will only lead to an hypertrophy of the remaining tissue, frequently to such an extent as to make the postoperative basal metabolic function unstable and, in most instances, of a higher magnitude than it was previous to the operation. This seems to be especially true of "high raters" manifesting the anxiety syndrome complicated by some sexual complex. To perform any surgical operation, except the one absolutely warranted and found necessary after a joint consultation of an internist, surgeon, and a competent psychiatrist thoroughly acquainted with the case under consideration, means to introduce into the patient's life new points of fixation, added causes for fear and worry, and hence, an increased demand upon the thyroid, resulting, as stated above, in a basal metabolic rate exceeding the one before the operation. These are not mere speculative statements, but are statements of facts which will be brought out more clearly in a following publication.

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## CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

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*(Continued from page 740.)*

### ROENTGENOGRAPHY AND FLUOROSCOPY IN CHILDREN

THE roentgen ray is an important aid in both the diagnosis and treatment of many diseases of infants and children. It is, however, necessary to know what to ask for from the roentgenologist, and to be able to interpret the plate after it is developed, and above all to correlate the roentgenological and the clinical pictures. The problem of keeping the child quiet during examination can, in part be overcome by rapid work.

#### THE NORMAL CHEST

The roentgenogram of a normal chest is subject to wide variations, especially with reference to the bronchial marking, hilus shadow and nodes of calcifications. Indeed it is likely that a real normal chest seldom, if ever,

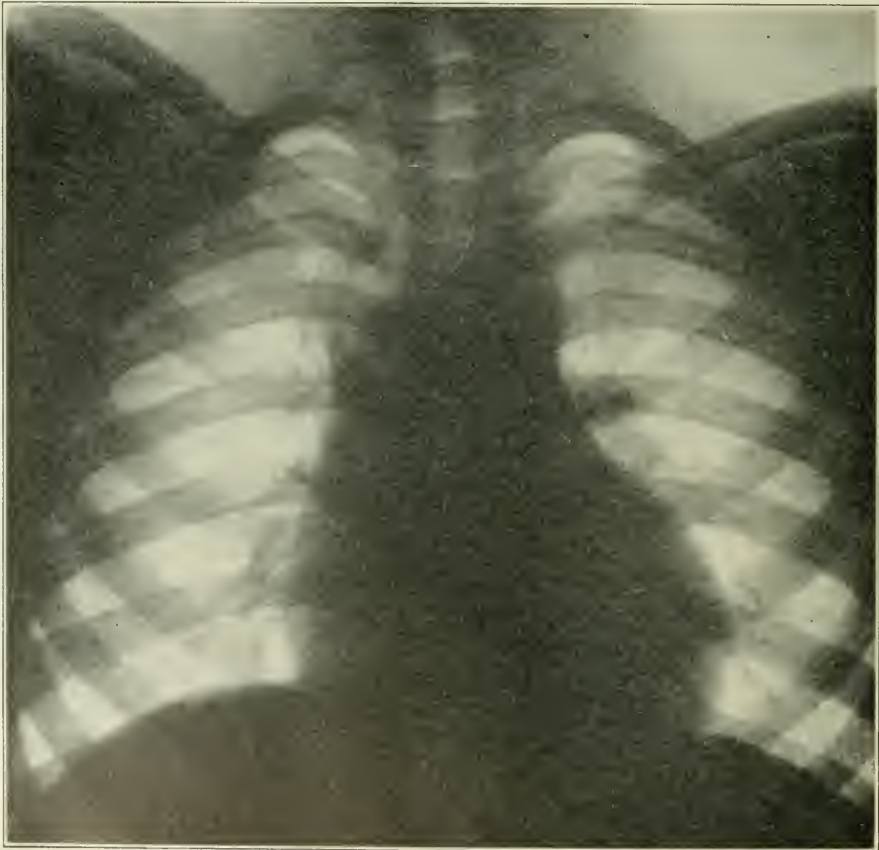


Fig. 32.—Chest of a child in good health showing some bronchial markings.

comes under observation, as any chest needing x-ray usually has some pathology in it to begin with. Furthermore, very few chests are clear of some infection or other. The term "normal" as generally used must therefore be considered as relative, rather than absolute.

A normal chest shows the outline of the heart, the lungs, the clavicle, the ribs, the diaphragm, and the scapula. An absolutely normal chest shows no areas of densities in the lungs. Some plates, however, show pronounced bronchial markings which fade before the outer third of the chest is reached. These children have usually gone through some previous lung infections

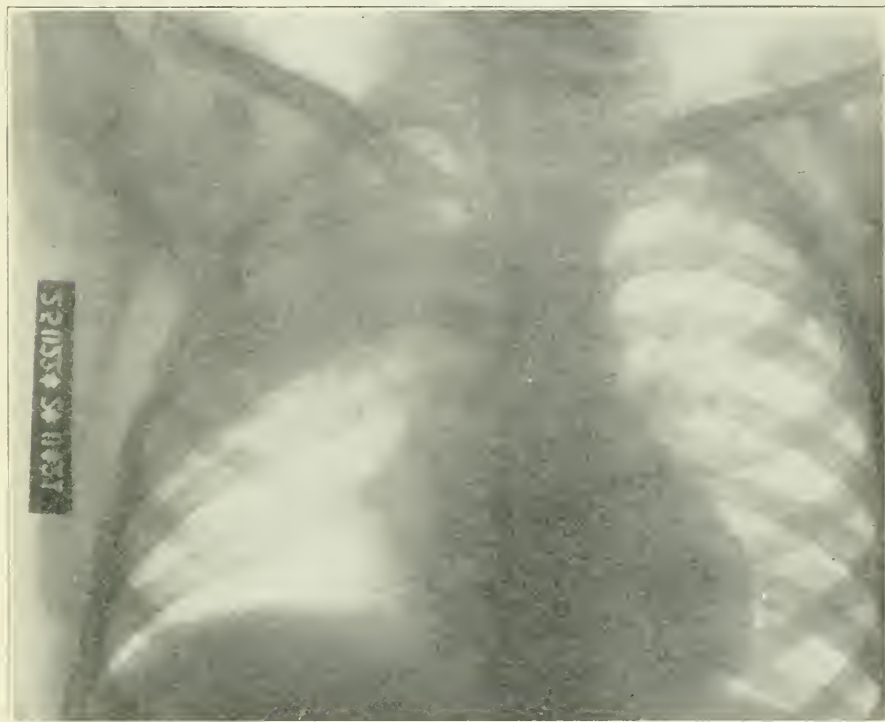


Fig. 33.—Lobar pneumonia. Consolidation of right upper lobe.

which left some bronchial markings (Fig. 32). No diagnosis of active pathology should therefore be made on these markings.

A hilus shadow, especially if it is subdivided into small areas, indicates an infection which may be tuberculous in character, but does not necessarily speak for an active infection, nor does it necessarily speak for a tuberculous process, as many other infections, especially influenza, may leave a hilus density. The same is true with calcified nodes. They are suggestive of a latent tuberculosis, but no diagnosis of this disease must be made merely on their presence.

#### DISEASES OF THE LUNGS AND PLEURA

Lobar pneumonia is characterized by a shadow of increased density of the lobe involved (Fig. 33). The structural markings are not seen as a rule.

The borderline between the consolidated and the unaffected portions of the lung is usually very sharp.

Bronchopneumonia may show only very little change in the plate. Both bronchitis and bronchopneumonia may show small areas of increased density along the bronchial tree variable in size and shape with rather indefinite outlines. These may occur anywhere, but are frequently found at the bases (Fig. 34).

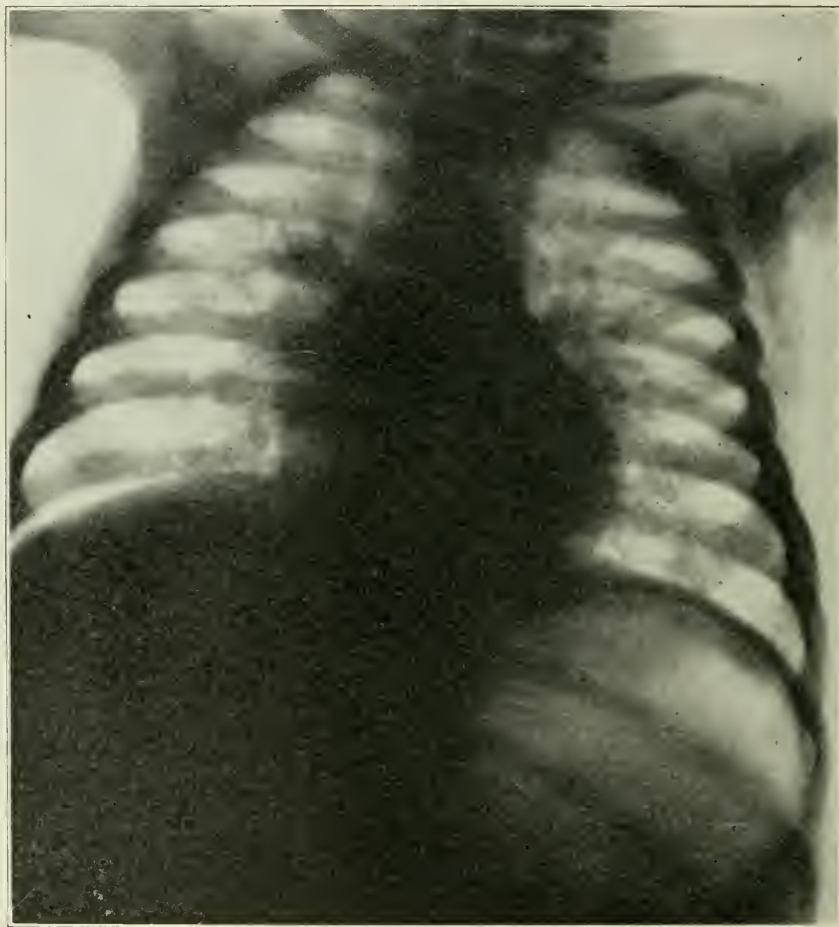


Fig. 34.—Bronchopneumonia.

Acute bronchitis may cause demonstrable roentgenographic changes. Chronic bronchitis may show increased markings followed well out into the periphery.

Tuberculous changes may be found anywhere in the lungs of children. The apical findings so frequently present in adults, however, are not common. Small shadows like a snowstorm scattered throughout the lungs are indicative of an acute miliary tuberculosis of the lungs (Fig. 35). The enlargement of the mediastinal shadow beyond the inner third of the chest speaks for enlarged tracheobronchial glands, which, in conjunction with

whispered bronchophony and positive tuberculin tests, speak for tuberculous glands, although no diagnosis of tuberculosis of tracheobronchial glands should be established on the hilus shadow.

Lung abscess is indicated by a clouding of the lung with a central necrotic area that is characterized by a dark area, of decreased resistance, surrounded by a dense peripheral zone.

Fluid in the chest cavity shows a shadow depending on the amount, on the location, and whether the fluid is free or encapsulated. Free fluid casts a shadow that may be seen to move upon change of position of the patient when viewed under the fluoroscope. It tends to seek a level. It may cause

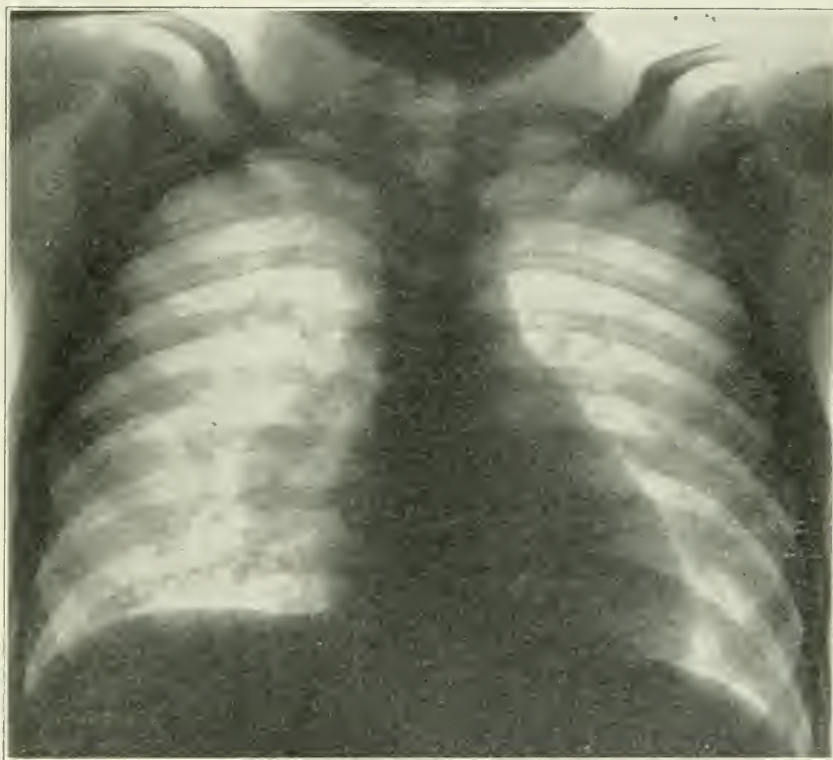


Fig. 35.—Miliary tuberculosis.

a dense shadow along the costal margin, and may push the lung in for a considerable distance. The diaphragmatic angle is obliterated, and the heart and mediastinum may be pushed to the opposite side. The shadow cast by fluid is more or less dense (Figs. 36-38), the structural markings gradually disappearing as the fluid becomes more and more of a thickened purulent character.

Encapsulated fluid may be seen in any part of the chest where there are opposing pleural surfaces. The outline of the fluid is quite definite, and the shadow is dense and free of lung markings. Thickened pleura may cast a shadow somewhat similar to a small amount of fluid.



Pneumothorax gives rather characteristic roentgenographic findings. The lung is compressed and pushed away from the thoracic wall. The air gives a transparent appearance to that portion of the chest, no structural markings are seen, and the edge of the collapsed lung is regular and sharply defined (Fig. 39).

Foreign bodies in the respiratory tract of children are of common occurrence. In relation to their examination by the roentgen ray, they may be divided into two classes: opaque and nonopaque. The nonopaque bodies in-

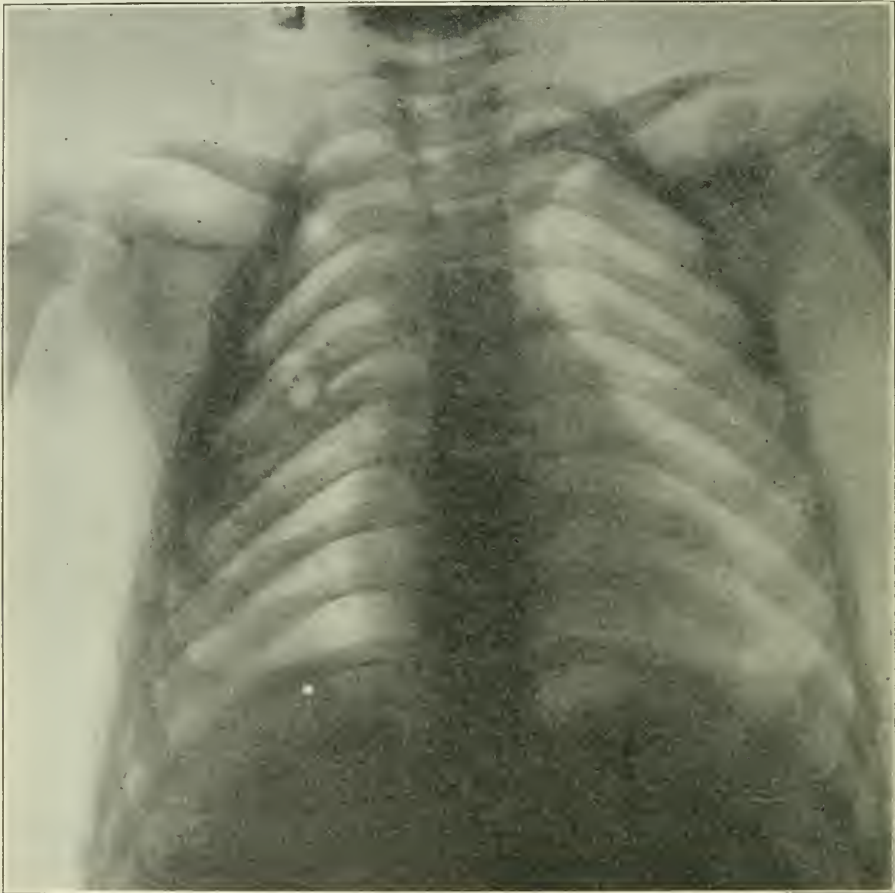


Fig. 36.—Postoperative pleural empyema on the right side, showing bridging of two ribs around drainage tube.

clude peanuts, beans, seeds, and nut shells. The opaque are chiefly metallic.

The radiographic findings vary, depending on the position and nature of the resulting obstruction. A monolateral emphysema results when the foreign body has passed the bifurcation, and is of such nature as to cause obstruction to the expiratory current. This is evidenced by the increased transparency of the affected area and depression of the diaphragm with partial fixation on that side. The heart and mediastinum are displaced away from the affected side, and there is increased excursion of the diaphragm

on the opposite side. When the foreign body completely obstructs the bronchus to ingress and egress of air, the residual air is quickly absorbed and atelectasis results. There is marked density of the shadow of the area involved. The heart and mediastinum are displaced toward the affected side. The diaphragm is retracted upward. Metallic objects cast a shadow and their detection is much less difficult.

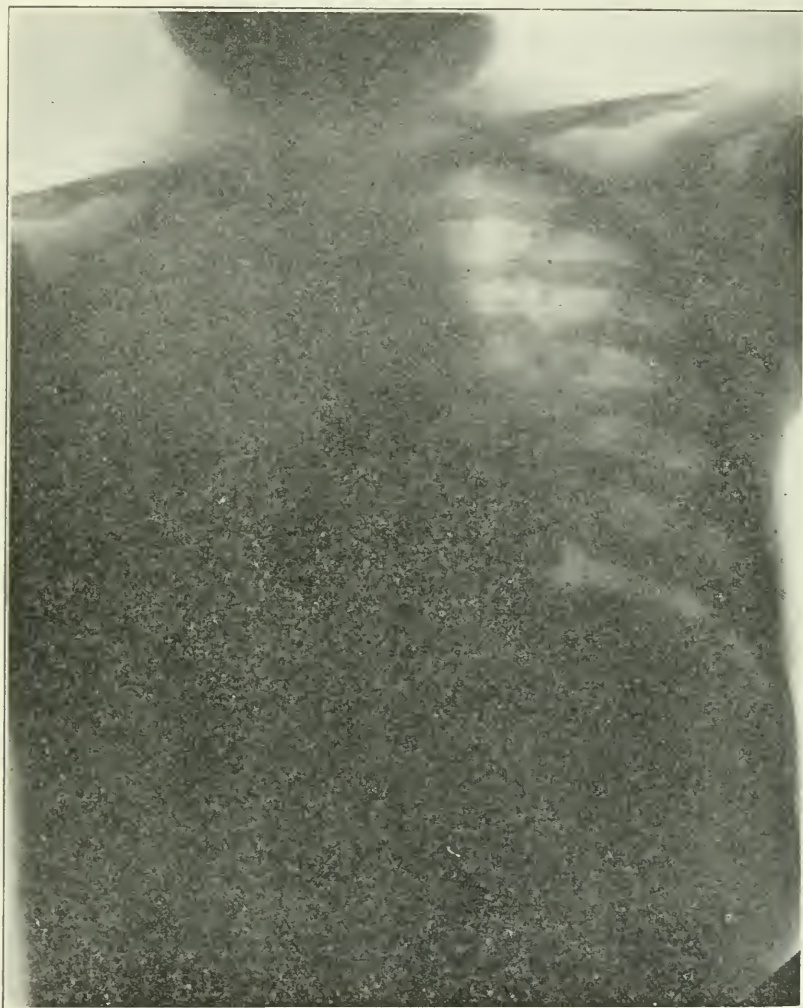


Fig. 37.—Empyema.

It is of the greatest importance to take roentgenograms at the end of full inspiration and at the end of expiration in order to show the above changes. The fluoroscope should always be used in the examination of these cases, and is of the greatest assistance in their removal.

#### HEART CONDITIONS

The heart shadow in children does not differ greatly from that of adults (Fig. 32). In infants the size of the heart in relation to the chest is increased.

Congenital hearts do not always show demonstrable findings. Often enlargement of the pulmonary artery may be demonstrated. Alterations in the normal heart curve indicate a pathologic condition of the heart (Fig. 40).

Pericardial effusion casts an increased shadow, which tends to be pear-

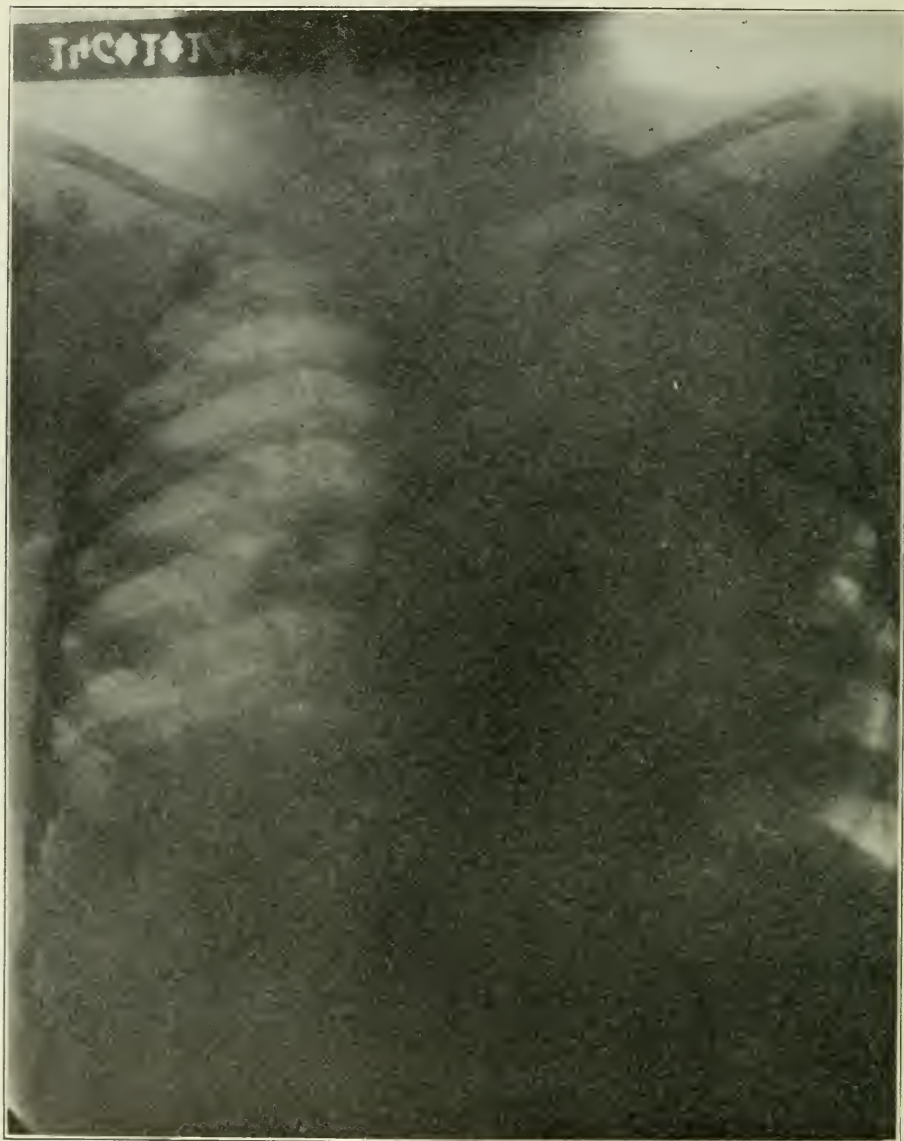


Fig. 38.—Encapsulated empyema (encysted).

shaped (Fig. 41). Cardiac enlargement is often demonstrated by the roentgenogram. The fluoroscope is used to advantage in both of these conditions. For actual measurement of the cardiac outline, distance plates are taken in order that the shadow cast may be of actual size.



## OTHER THORACIC CONDITIONS

Persistent thymus may be demonstrated by a homogeneous shadow extending downward from the clavicles on either side of the midsternum (Fig. 42). The width and extent of this shadow depend on the size and shape of the thymus.

The roentgen ray has also been found to be effective in the treatment of these cases. Overexposure, however, is to be avoided, because of the danger of skin burns, deleterious effect on the thyroid, and too rapid atrophy of the thymus.

Thoracic tumors are rare. Suffice it to mention the dense shadow which they cast, and the frequency of their origin from the mediastinum.



Fig. 39.—Right pneumothorax with complete collapse of lung.

## GASTROINTESTINAL CONDITIONS

In pyloric stenosis of infancy, and in other cases of persistent vomiting, the diagnosis may be expedited by following the course of an opaque meal, as shown by the fluoroscope and roentgenogram. The large dilated stomach, the thickened wall, the hyperperistaltic waves, and the reversed peristalsis may all be demonstrated. The percentage of the bismuth meal that passes the pylorus in a given time is indicative of the patency of the pylorus (Fig. 43). When one-half to two-thirds of the meal passes the pylorus in three hours, the immediate need of surgical interference is not indicated, and the condition, at most, is one of partial obstruction. If the bismuth is entirely re-



tained in the stomach after 4 hours there is complete obstruction of the pylorus and operation is indicated. Bismuth subcarbonate and barium are the salts most frequently used. They may be given in a small feeding in amounts of one-half to one ounce shortly before the examination is begun.

The information obtained by the observation of an opaque enema, by means of the roentgen ray, is of importance in cases of dilated colon and

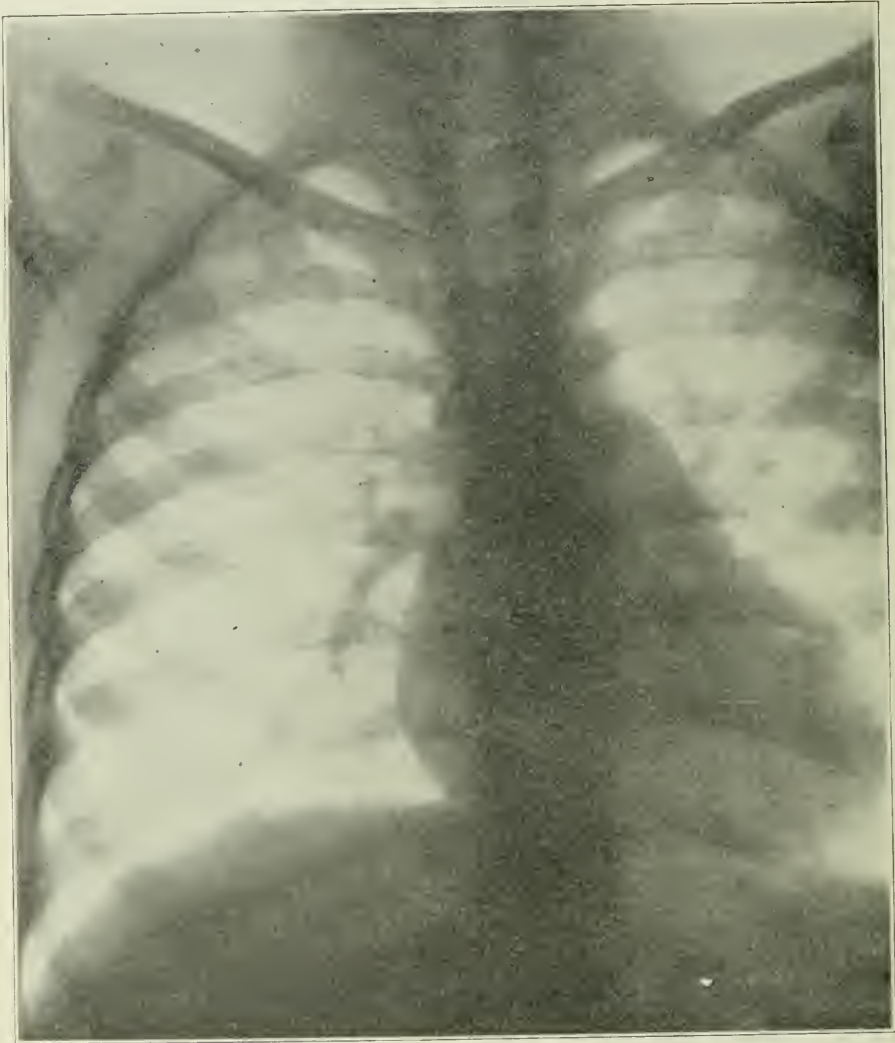


Fig. 40.—Pathological heart showing projection of left pulmonic curve.

Hirschsprung's disease. The increased diameter, looping, and any increase in length may be seen. Congenital malformation may be demonstrated, but not often. Tumors of the intestinal tract, as well as other tumors in the abdomen, may be seen at times if they are of sufficient size and density.

Calculi in the kidneys, ureters, and bladder may be shown in the roentgenogram. The examination of the urinary tract by means of opaque in-

jections may be done, but it is not frequently carried out because of the added dangers and difficulty of the operation. Dilatation of the pelvis of the kidney, dilatation of the ureters, and diverticula of the bladder may be demonstrated by this procedure.

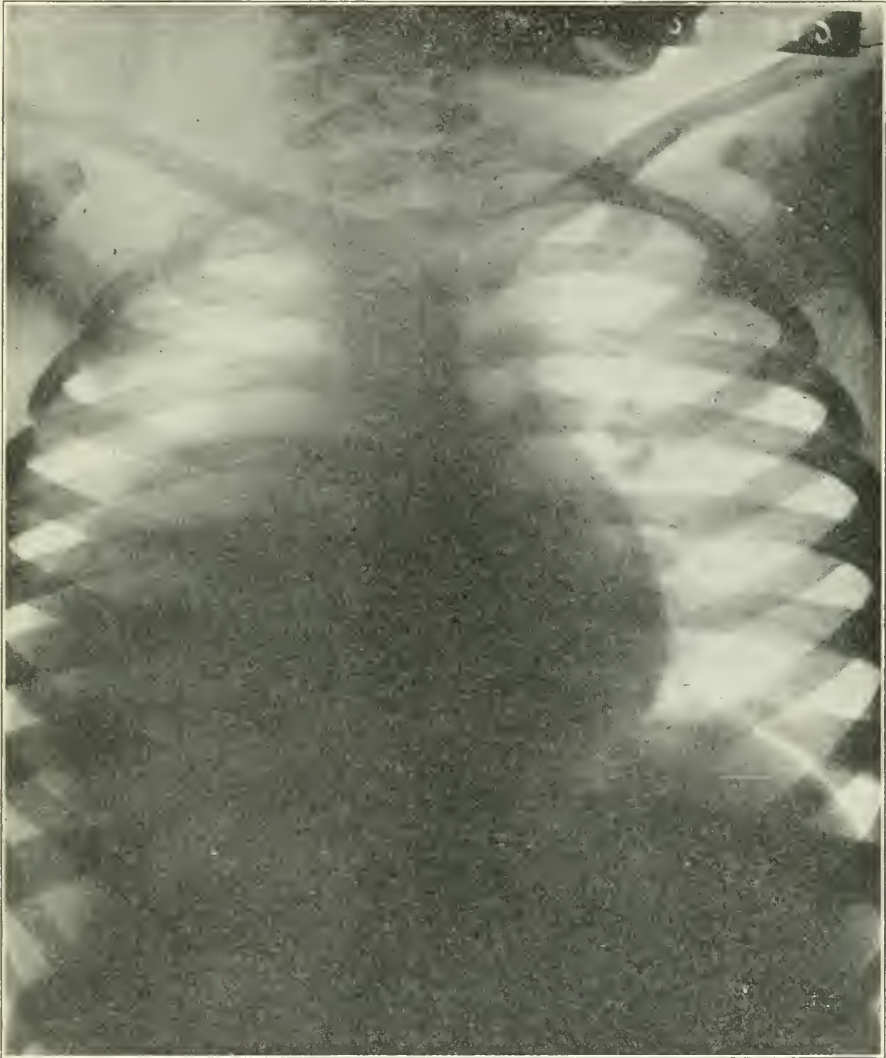


Fig. 41.—Precarditis with effusion.

The roentgen ray examination of the mastoid, nasal sinuses, and teeth is of service in older children. The late development of the sinuses in children, makes their examination less frequently necessary.

#### BONES AND JOINTS

Tuberculosis of the bone may be said to involve the epiphyses. Tuberculosis of the shaft is seen, but is rare. Tuberculous dactylitis is frequently seen

in tuberculous children and it may be impossible to differentiate the lesion from that of lues or osteomyelitis. Syphilis tends to involve more than one bone, and bone production is increased. Periostitis, and even tertiary lesions are often demonstrable in the roentgenogram. The serological tests and clinical course help to establish a definite diagnosis.

The most important differential points in the diagnosis of chronic bone and joint conditions in children may be summarized as follows:

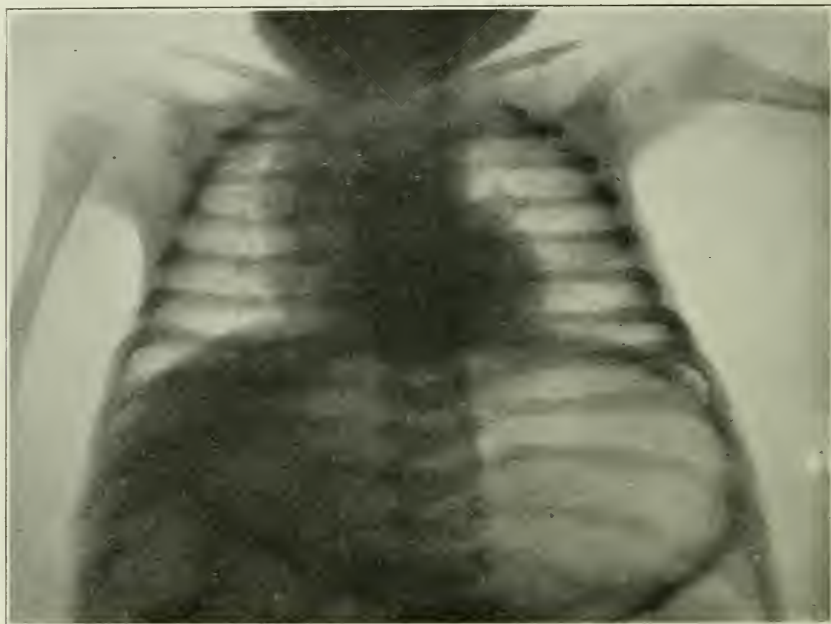


Fig. 42.—Persistent thymus. Broad superior mediastinum.

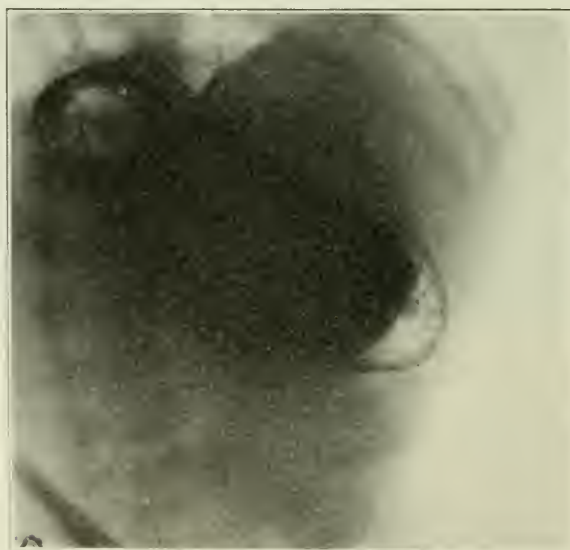


Fig. 43.—Pyloric stenosis showing gastric retention after four hours.

## RICKETS.—

- a. Multiple involvement.
- b. Early the diaphysis is frayed out and no longer clear cut.
- c. This becomes more marked, and there is a dense shadow cast by the margin, which becomes wider, and has an inverted saucer-shaped appearance (Fig. 44).
- d. Atrophy is often present, and fractures not uncommon.
- e. In old cases, dense, transverse, linear shadows may be seen in the diaphysis, indicating a nutritional disturbance when the epiphysis was at that point.

## CONGENITAL SYPHILIS.—

- a. No spreading out of the epiphyseal line.
- b. No atrophy.
- c. Periostitis usually present and extensive. It is laminated and is usually found on the lower third of the fibula and radii.

## SCURVY.—

- a. Epiphysis and epiphyseal line undisturbed.
- b. "White line," 1-2 millimeters behind the epiphyseal line, extending over the entire bone outline (Fig. 45).
- c. Subperiosteal hemorrhage nearly always present but may not be seen in the skiagraph.
- d. Epiphyseal separation may be present.

## TUBERCULOSIS.—

- a. Most often confined to one joint.
- b. Cartilaginous destruction is noted by the narrowing of the joint space.
- c. Irregular, worm-eaten appearance, and general cloudiness.

## PERTHES' DISEASE.—

- a. Limited to the hip joint.
- b. No cloudiness, bone detail clear cut.
- c. The epiphysis, seems more flattened, denser than normal, and is not eroded or worm-eaten.

CHRONIC ARTHRITIS (Still's Disease).—The roentgenograph shows a thickening of the periarticular tissues, often distention of the joint. Osteoporosis may be noted.

OSTEOGENESIS IMPERFECTA.—There is decreased bone density; shortening of the long bones is present. Multiple fractures are nearly always present.

CHONDRODYSSTROPHY.—There is marked shortening of all of the long bones. These give normal or increased shadows. The shortening is so marked as to cause little difficulty in the recognition of the condition.

Bone infections are frequent in children. Osteomyelitis is frequently acute. It is characterized by rapid bone destruction without bone regeneration (due to its rapidity).

The clinical findings may be such that a positive diagnosis can be made before any changes are seen in the roentgenogram. The earliest findings may



be seen as areas of decreased density in the medullary cavity; later, when the infection has traveled into the cortex, areas of decreased density may be demonstrated. In less acute and in chronic cases, bone destruction and regeneration may be seen (Fig. 46); still later, the sequestrum may be shown.

**BONE INJURIES.**—Epiphyseal separation and injuries are frequent. The epiphyses at birth are for the most part cartilaginous, and are, therefore, not seen

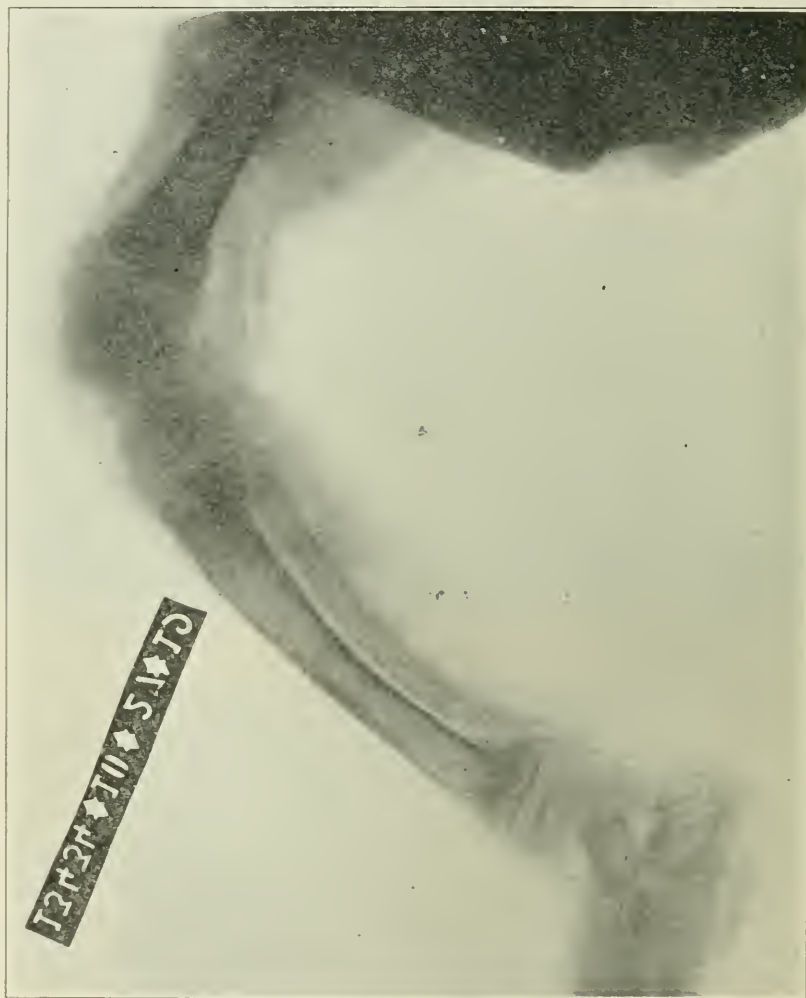


Fig. 44.—Rickets, showing flaring out of ends of the long bones.

in the roentgenograph. Nutritional disturbances delay the appearance and development of the epiphyses. In roentgen ray examination it is of the greatest importance to know the time of the appearance of these ossification centers.

In children, when the force of an injury centers at the epiphysis, epiphyseal separation occurs more often than fracture. Fractures in children are often of the green-stick variety, due to the tendency of the bones to bend. The

frequency of epiphyseal injury in children, and the difficulty in their recognition, both clinical and by the roentgen ray, must be kept in mind in the diagnosis and treatment of injuries about the joints. Corresponding parts should always be examined, and the two compared, in order to determine the extent of the injury. Antero-posterior, and lateral skiagraphs should be taken. The parts to be examined should be in close proximity to the film, or plate, in order to be as little distorted as possible.

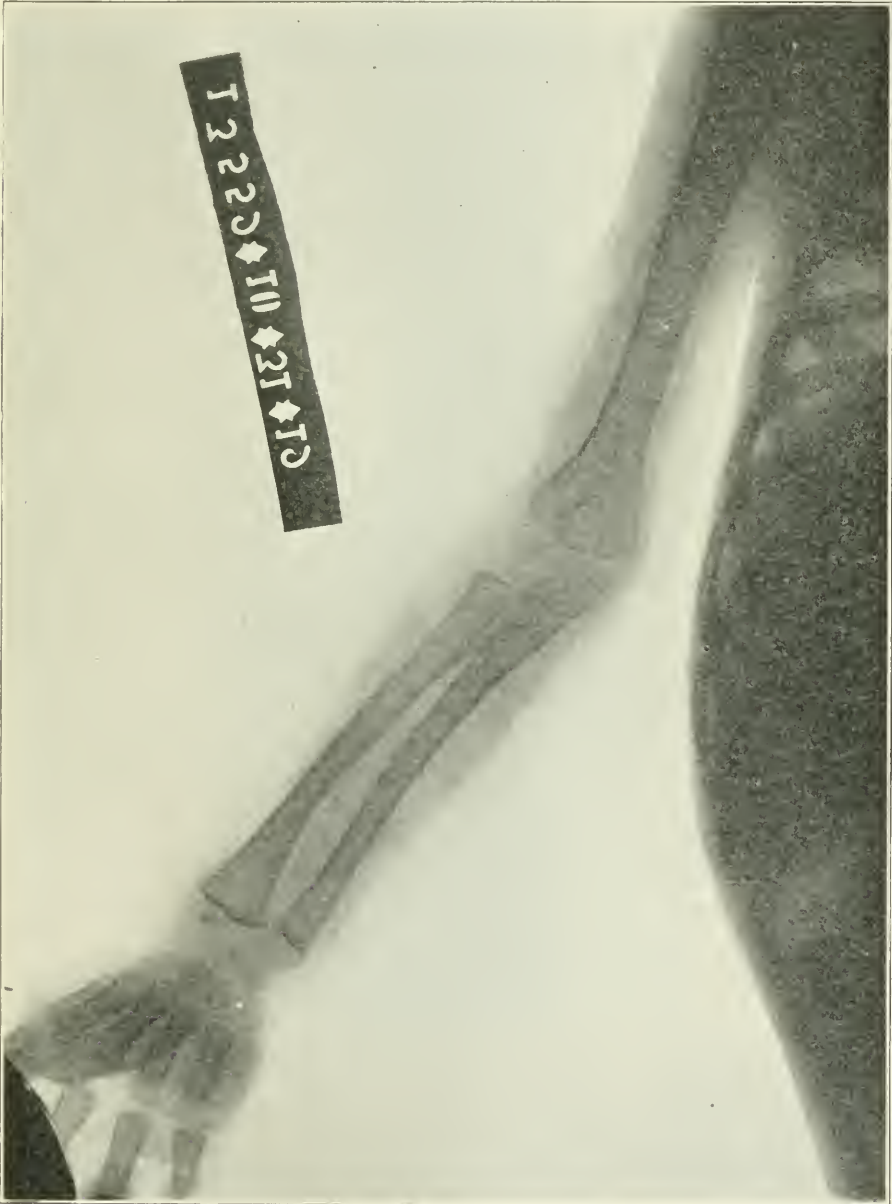


Fig. 45.—Scurvy showing the characteristic white line bordering the long bones (the line shows black in reproduction).

Of the congenital dislocations the hip and shoulders predominate. Congenital dislocation of the hip is often unnoticed until the child begins to walk, and it may be some time before any notice is taken of the waddling gait. In the roentgenogram, the shape of the pelvis, and the position of the head and neck of the femur in comparison with the normal side may make the diagnosis



Fig. 46.—Osteomyelitis of tibia with almost complete destruction.

clear. The acquired dislocations usually occur in later childhood, and the findings are similar to adult dislocations.

The roentgen ray is useful after reduction of fractures and dislocations has been made to determine the correctness of the parts as to apposition and alignment.

TABLE XIV  
DEVELOPMENT AND UNION OF SOME OF THE MORE FREQUENT EPIPHYSES

LOCATION		APPEARANCE	UNION
Humerus, head		6-8 mo.	} fuse at about 6 yr. 17-18 yr.
trochanters		3-4 yr.	
capitellum		1 yr.	
Internal condyle		5 yr.	
trochlea		10-11 yr.	
External condyle		12-14 yr.	
Radius			
Lower epiphysis		2-3 yr.	17 yr.
Olecranon		8-9 yr.	17-18 yr.
Femur, head		2-3 yr.	17-18 yr.
Lower epiphysis		at birth	18-20 yr.
Tibia, lower epiphysis		2-3 yr.	17-18 yr.

*(To be continued.)*



# INVESTIGATIONS ON THE DOUBLE TYPE OF THE RECEPTORS IN THE TYPHOID-PARATYPHOID GROUP AND THEIR SIG- NIFICANCE FOR THE WIDAL REACTION\*

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INVESTIGATIONS of recent years have shown that bacteria,<sup>1</sup> as well as other cells, for example sheep's erythrocytes,<sup>2</sup> may function as antigens through the simultaneous participation of a multiplicity of chemically distinct receptor groups. Apart from such investigations of *chemical* differences, Joos<sup>3</sup> found in many bacteria, particularly typhoid bacilli, two *serologically* differentiable agglutinogens. He separated them by heating to 60° C. to 62° C., at which temperature one of these agglutinogens was destroyed, while the other was unaffected by subjection to it for even several hours. Joos also noted that the flakes produced by the agglutination of the thermolabile antigen were coarse while those resulting from the thermostable fraction were fine. These investigations of Joos did not attract the attention they deserved, probably largely because heating to 62° C., as he recommended, is often insufficient to destroy the thermolabile agglutinin.

It was first the studies of Weil and Felix<sup>4</sup> and their coworkers that showed the importance of closer analysis of the agglutination reaction than had previously been made. Working with proteus bacilli, they succeeded in serologically delimiting two proteus groups which they had isolated from typhus patients (the so-called X-strains) from one another and from the vulgar proteus strains. Weil and Felix found that the X2 and X19 strains have identical *thermolabile* but *different thermostable* receptors. Consequently, after destroying the thermolabile receptors by heating, it is immediately possible to serologically differentiate these two strains, though differentiation by an immune serum cannot be effected on bacilli with intact thermolabile receptors.

As a result of these investigations of Weil and Felix, and the subsequent ones of Schiff,<sup>5</sup> H. Sachs,<sup>6</sup> and others, it is now well established that in the typhoid, paratyphoid, Gärtner, and proteus groups, there are two groups of receptors, one of which is destroyed by heating to 100° C. for an hour, while the other is not affected by even a longer subjection to this temperature (in our experiments we have often heated to 100° C. for more than two hours without damage to the stable receptors). The agglutination of the thermostable antigen occurs in *fine* compact flakes which on sinking to the bottom of the tube form a compact sediment. On the other hand the action of the specific agglutinins on the thermolabile receptors results in *coarse*, rather loose flakes which form a bulky loose sediment, so that, in most

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cases, with a little practice one can tell from the appearance of the agglutination whether the thermolabile or the thermostable antigen is being carried down. For this purpose a hand lens is usually necessary, though a so-called agglutinoscope (Kuhn and Woithe) is preferable. When both types of flocculi are present in the solution, simple inspection often does not suffice, and recourse must be had to absorption experiments (see below). We have found the time factor a great aid in differentiation. The coarse agglutination occurs much more rapidly than the fine, being usually complete (with typhoid bacilli) in less than half an hour. On the other hand the fine agglutination may require several hours to attain completion. It is not uncommon in following the progress of an agglutination reaction to initially observe what is apparently a purely coarse agglutination which is later complicated by the appearance of fine flakes also.

Analysis of the receptor apparatus in the typhoid-paratyphoid-Gärtner group by means of absorption experiments on artificial immune sera has been carried out by Weil and Felix, Schiff, and others. We have also made such experiments, using the stock strains in the laboratory, and have arrived at essentially the same results, which are as follows: The three varieties of bacteria have thermostable receptors in common, but the thermolabile receptors are specific. The thermostable receptors of typhoid and Gärtner bacilli are qualitatively identical. Paratyphoid B has thermostable receptors which are identical with those of the other two but has also specific receptors of this type. The following schema indicates these relationships:

	THERMOSTABLE RECEPTORS	THERMOLABILE RECEPTORS
Typhoid	AB	a
Paratyphoid B	AC	b
Gärtner	AB	c

To the thermolabile and thermostable agglutinogens correspond two different agglutinins, designated by Schiff as labilotropic (the coarse-flaking) and stabilotropic (the fine-flaking) agglutinins. In an ordinary typhoid immune serum both agglutinins are present, but their quantitative relationships may vary greatly. As a rule, in the highest dilutions still causing agglutination the effect is due to the labilotropic antibodies; i.e., the labilotropic end titer is higher than the stabilotropic. Rather uncommonly the reverse is true. Purely fine-flaking typhoid immune sera can be obtained by immunizing with typhoid bacilli that have been heated to 100° C. sufficiently long to destroy the thermolabile agglutinogens. Or such a serum can be prepared from an ordinary immune serum containing both types by absorbing the stabilotropic agglutinins with bacilli heated to 100° C. for an hour or more.

As yet the thermolabile agglutinogens have been found only in bacteria with well developed flagella. It has, therefore, been suggested that the thermolabile receptors are contained in the flagella, or at least intimately associated with their presence.<sup>7</sup> Hence the agglutination type is of no significance for the diagnosis of infections with dysentery bacilli, the cocci, etc., but it might very well be supposed, *a priori*, that it would be worthy of

consideration in the agglutination test for typhoid fever. I have, therefore, with the kind cooperation of Dr. Fritz Schiff, Director of the Bacteriological Institute of the Krankenhaus im Friedrichshain, Berlin, investigated the types of agglutinins in the sera of typhoid and paratyphoid patients and their importance for the diagnosis of the disease.

The following were the points investigated:

- (1) To which type the agglutinins of normal serum belong.
- (2) The type of the agglutinins in the sera of typhoid patients.
- (3) The significance of the receptor types for the technical details of the Widal reaction.
- (4) The significance of the agglutination type for differential diagnosis.
- (5) The type of the agglutinins in the paratyphoid infections.

#### (1) THE AGGLUTININS OF NORMAL SERA

It is a well known phenomenon that typhoid bacilli are sometimes agglutinated by the serum of individuals who have neither typhoid fever at the time of the examination nor a history of a previous typhoid infection or vaccination. In the course of our investigation we studied 60 normal sera. About 20 per cent of these sera in concentrations of 1:20 agglutinated typhoid bacilli. In three cases the agglutination was still present at a dilution of 1:50. These results were obtained when the reaction was read after two hours: if the reading was carried out after twenty-four hours most of the normal sera caused at least slight agglutination in the very high concentrations. But in every case the agglutination was in *fine flakes*, that is, *the normal agglutinins are stabilotropic* (fine-flaking), and can be completely absorbed from the serum by typhoid bacilli which have been heated to 100° C. for one hour, as is shown, for example, in the following protocol:

Serum 1083, diagnosis syphilis of the liver. A 24-hour agar plate culture of typhoid bacilli was suspended in physiological salt solution and heated on the water-bath to 100°C for one hour, centrifuged, the supernatant fluid poured off, and 5 c.c. of 1:10 dilution of the serum added to the sediment. After mixing, it was put in the incubator at 37°C for 15 minutes, again centrifuged, the supernatant serum poured off and used for the agglutination. All subsequent absorption experiments were carried out similarly. But in doing such experiments it should be borne in mind that complete absorption from strong, concentrated sera is difficult, so that it is better to carry out the absorption on higher dilutions of concentrated sera, and repeat it once or even twice.

SERUM DILUTION	UNTREATED SERUM WITH		ABSORBED SERUM WITH	
	Living typhoid bacilli	Typhoid bac. heated to 100° 1 hour	Living typhoid bacilli	Typhoid bac. heated to 100° 1 hour
1:10	++ fine	+ fine	—	—
1:20	+ fine	(+) fine	—	—
1:50	(+) fine	(+) fine	—	—
1:100	—	—	—	—
1:250	—	—	—	—

Reading after two hours. Plus signs in parentheses mean that the agglutination could only be determined with certainty with the aid of the agglutinoscope.

All our other analyses of agglutinating sera from nontyphoid patients gave similar results, so the following conclusions seem justifiable:

(1) A coarse agglutination is not due to the normal agglutinins.

(2) If the agglutinins for typhoid bacilli cannot be removed from a serum by absorption with these bacilli heated to 100° C. for one hour (observing the precautions, with concentrated sera, mentioned above), the agglutination is not due to normal agglutinins. But the converse is not necessarily true, i.e., a purely fine agglutination may be brought about by the serum of a typhoid patient (see below).

(3) Since stabilotropic (fine) agglutination occurs much more slowly than labilotropic, normal sera are far more likely to give an apparently positive agglutination test if the reading is done after twenty-four hours than if carried out after one or two hours.

Of four icteric sera (which are often stated to give quite frequent positive Widal's,) one gave a positive reaction in a dilution of 1:50. It was typically stabilotropic (fine), and as a matter of fact there was no indication of typhoid in the history.

## (2) THE AGGLUTININS IN TYPHOID FEVER

We investigated the sera of 31 patients suffering from typhoid fever of all stages. Their content in stabilotropic agglutinins was determined by

TABLE I

SERUM	LABILOTROPIC TITER	STABILOTROPIC TITER
2144	1:1000	1:2000
71 V	1:250	1:250
B 41	1:1000	1:100
2284	1:2500	1:500
2184	1:200	1:20
2206	1:1000	1:250
2255	1:2500	neg. in 1:50
2252	1:2500	1:100
2258	1:2500	1:400
3083	1:500	1:250
3121	1:500	1:100
3209	1:50	neg. in 1:10
3212	1:1000	1:100
3215	1:2500	1:1000
3220	1:250	1:50
3221	1:250	neg. in 1:10
3228	1:100	1:10
3254	1:250	1:50
3258	1:500	1:100
3226	1:750	1:50
3180	1:100	1:1000
3197	1:100	1:50
3203	1:250	neg.
3232	1:100	1:10
3240	1:500	1:1000
2266	1:1000	neg.
2179	1:200	neg. in 1:10
2283	1:2500	1:250
R	1:250	1:50
2068	1:5000	1:5000
3126	1:1000	1:25

Reading in all cases after 24 hours.



agglutination experiments with typhoid bacilli heated to 100° C. or with Gärtner bacilli, whose thermostable receptors we have seen to be identical with those of the typhoid bacillus. The labilotropic titer could be taken as identical with the titer of the entire serum when the latter was higher than the stabilotropic titer. In cases of doubt it was determined by first absorbing the stabilotropic agglutinins with heated bacilli. Table I shows the titer of the sera of typhoid patients in both types of agglutinins:

From this table we can see that while all the typhoid sera contained labilotropic agglutinins, six (almost 20 per cent) were lacking in the stabilotropic type. In but three cases was the stabilotropic titer the higher, in two the titers were equal, while in the remaining 26 the labilotropic titer was the higher.

In a case which was studied from an early stage (before the Widal reaction became positive) to death, no relation of the agglutinin type to the stage of the disease could be discerned.

### (3) SIGNIFICANCE OF THE RECEPTOR TYPES FOR THE TECHNICAL DETAILS OF THE WIDAL REACTION

(a) *Time of Reading.* Labilotropic agglutination occurs very rapidly, so that sera in which these agglutinins predominate (as they do in the large majority of cases) can be read very soon. Agglutination of this type is usually almost completed within half an hour, and practically always within two hours. On the other hand, if the serum contains largely stabilotropic (fine) agglutinins, agglutination may not be complete for many hours, so that reactions negative at the end of two hours should always be seen after 24 hours. But in interpreting these late (almost always predominantly fine) agglutinations, it should always be recalled that the normal and the partial agglutinins are fine. Agglutination present at half an hour is, on the contrary, in the large majority of cases coarse and therefore almost surely specific.

(b) *Selection of the Strain.* Weil and Felix<sup>8</sup> analyzed the receptors of many strains of typhoid bacilli and found little variation in the thermolabile receptors of the different strains, but very considerable fluctuations in the agglutinability of the thermostable receptors; some strains showed practically no stabilotropic (fine) agglutination. Hence, in the selection of a strain for routine Widal reactions, care should be taken to obtain one with functioning thermostable receptors, or positive agglutinations with sera whose agglutinins are largely stabilotropic may fail of detection. The thermostable receptors of a strain are tested by heating the bacilli to 100° C. for one hour and then seeing how well they are agglutinated by an artificial immune serum known to contain both types of antibodies.

(c) *Use of a "Typhoid Diagnosticum."* The diagnosticum must be prepared so that it contains both types of receptors. We have tested the Ficker Diagnosticum, which is widely used, and found that it does contain both varieties of receptor. In our experiments we have found, as did Weil and Felix, that alcohol destroys the labilotropic agglutinins, and therefore typhoid

bacilli which have been treated with alcohol for 24 hours and then suspended in salt solution act as do heated bacilli, and may be used for the detection of the stabilotropic agglutinins alone.

#### (4) SIGNIFICANCE OF THE AGGLUTINATION TYPE FOR DIFFERENTIAL DIAGNOSIS

In carrying out the Widal reaction it not rarely occurs that the end-titer of the serum for typhoid and paratyphoid B. is so nearly the same that it is impossible to say, in the absence of a positive culture, which is the infecting organism. Consideration of the agglutination type may be of great aid in such a dilemma. If early inspection (after 10 to 15 minutes at 37° C.) of the tubes containing typhoid bacilli shows a labilotropic (coarse) agglutination, the infecting organism is the typhoid bacillus for, as we have seen, the normal agglutinins are stabilotropic and the typhoid-paratyphoid group have in common only some stable receptors. But it is to be emphasized that absence of coarse agglutination with typhoid bacilli does not absolutely exclude a typhoid infection for there are rare typhoid sera containing almost exclusively stabilotropic (fine) agglutinins, though we did not encounter in our series of 31 cases, any totally lacking in stabilotropic antibodies. Naturally, in paratyphoid infections the coarse agglutination is found in the tubes containing paratyphoid bacilli, the agglutinins for typhoid bacilli, if any, being fine-flaking.

For purposes of differential diagnosis in the typhoid-paratyphoid infections Castellani's absorption experiment is used considerably, but often fails to attain its goal. The conditions for its success or failure can be explained by consideration of the receptor types. In the typhoid-paratyphoid Gärtner group the thermolabile (coarse) receptors are, with rare exceptions, specific, while many of the thermostable (fine) receptors are possessed in common by the three varieties of bacteria, in fact the thermostable receptors of typhoid and Gärtner bacilli are entirely identical. Hence, differentiation of sera from typhoid and Gärtner bacillus infections by means of absorption experiments is only possible when the labilotropic agglutinins are present in sufficient concentration. The Castellani absorption method will differentiate paratyphoid infections when the labilotropic agglutinins are abundant, but not always when the stabilotropic predominate.

#### (5) THE AGGLUTININS IN THE SERA OF PARATYPHOID B. INFECTIONS

The sera of four patients suffering from paratyphoid B. infections were investigated. All of these infections were of the typhoid form, there being no sera of cases of gastro-enteritis due to paratyphoid bacilli. These sera were tested as to their ability to agglutinate paratyphoid B. bacilli, paratyphoid B. bacilli that had been heated to 100° C. for one hour, typhoid bacilli, and the Aertryck strain of paratyphoid B., which is a cause of infectious meat poisoning. Similar tests were carried out on the sera after their stabilotropic agglutinins had been removed by absorption with paratyphoid B. bacilli that had been heated to 100° C. for one hour. Table II shows the results:

TABLE II

SERUM OF PATIENT	TITER OF UNTREATED SERUM FOR			
	Paratyph. B	Heated Paratyph. B.	Aertryek	Typhoid
Gr.	1:1000 c. & f.	1:250 f.	1:250 f.	1:1000 f.
Ch.	1:500 c. & f.	1:50 f.	1:250 f.	1:100 f.
B.	1:2500 c. & f.	1:1000 f.	1:500 f.	1:500 f.
K.	1:250 c. & f.	1:10 f.	neg.	neg.
	Titer of serum after absorption:			
Gr.	1:500 c.	neg.	neg.	neg.
Ch.	1:500 c.	neg.	neg.	neg.
B.	1:2500 c.	neg.	neg.	neg.
K.	1:100 c.	neg.	neg.	neg.

c = coarse, f = fine. Reading after 24 hours.

The above results demonstrate that the sera of these four patients infected by paratyphoid B. contain both labilotropic and stabilotropic agglutinins for the infecting organism, but that the partial agglutinins are fine and can be removed from the serum by absorption with heated paratyphoid B. bacilli. These experiments also confirm the previous results of Schiff, who found that the ordinary strains of paratyphoid B. bacilli differ from the strains causing acute gastroenteritis (Breslau, Aertryek, etc.) in their thermolabile receptors, though the thermostable fractions are identical. So that to the striking differences in the clinical courses of the two main groups of paratyphoid B. infections there correspond definite serological differences between the respective infecting organisms.

I should like to express my thanks to Privat Dozent Dr. Fritz Schiff for many courtesies extended in the course of this investigation, as well as to the Robert Koch Institute and the Bacteriological Laboratories at Kiel and Greifswald for sera kindly placed at my disposal.

#### RECAPITULATION

1. The receptors of the typhoid-paratyphoid-Gärtner group are of two types—thermolabile and thermostable. To each agglutino-gen corresponds a specific agglutinin, termed respectively labilotropic and stabilotropic.

2. Agglutination of the thermolabile antigen occurs in coarse flakes, of the thermostable in fine flakes.

3. When typhoid bacilli are agglutinated by the serum of a patient who has never had typhoid fever, the agglutination is of the fine type.

4. Partial agglutination in the typhoid-paratyphoid-Gärtner group is of the fine type.

5. All our sera giving positive Widal reactions—31 in number—contained labilotropic agglutinins, 26 of the sera also contained stabilotropic agglutinins.

6. We did not encounter any coarse agglutination reactions brought about by sera of other than typhoid patients. The “nonspecific Widal’s” showed the fine type of agglutination.

7. Consideration of the receptor type is of significance for the technical details of the Widal reaction, particularly the time of reading and the choice of the strain employed, as well as for the Castellani experiment.

8. Sera from patients suffering from the typhoidal form of paratyphoid-B. infection contain both stabilotropic and labilotropic agglutinins for the usual strains of paratyphoid-B. bacilli, but only stabilotropic agglutinins for typhoid bacilli and for these strains of paratyphoid-B. (*Aertryck*, etc.) which cause acute gastroenteritis.

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## BACTERIOPHAGE PHENOMENA. II\*

BY LLOYD ARNOLD, M.D., CHICAGO, ILL.

WE have called attention in a previous paper in this journal<sup>5</sup> to the increased activity of a bacteriophage substance when added to a 3 hour old culture of a susceptible microorganism. This increase in virulency is very striking, leading in the majority of instances to complete sterilization of the culture. The same bacteriophage added in the same concentration to cultures of varying ages, ranging from 1 to 12 hours old, does not produce so marked a change as it does in the 3 hour old culture. In some few instances this takes place in either the 2 or the 4 hour old cultures, but usually it is found in the 3 hour old culture.

The life cycle of a bacterial culture may be conveniently divided into: (a) latent period; (b) period of maximum rate of growth or the logarithmic period; (c) stationary period; (d) period of decline. These phases are determined by estimations of the number of viable microorganisms present at various intervals. It is well known that these periods merge into one another without a sharp dividing line. The duration of these phases vary with the different species of bacteria, and even with the same microorganism under different environmental conditions.

By the latent period or lag is meant the interval which elapses between the time of inoculation and the time at which maximum rate of growth begins. The period was first recognized by Muller.<sup>1</sup> This varies from about 1 to 3 hours in duration, depending upon whether the bacteria have been accustomed to grow upon the medium used; the temperature, and upon other factors as yet unknown.

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The logarithmic period, is the phase of rapid growth during which the bacteria are dividing regularly; they increase in number in a standard unit of time, following the law of geometric proportion. This period varies from 2 to 8 hours in duration (Rahm,<sup>2</sup> Penfold,<sup>3</sup> and Chesney<sup>4</sup>).

The stationary period, is the phase in which the bacteria cease to multiply at a maximum rate; their increase in number becomes less and finally ceases. The duration of this period is much longer in time than either of the two previously mentioned phases. After expiration of several days, the period of decline begins, in which the number of living bacteria decreases.

After a short period of rest, the lag phase, there begins a logarithmic or maximum growth period; this can be thought of as a stimulation period. It is during this period that the bacteria multiply by geometric progression. It is during the beginning of this stimulation period that we find the bacteria most susceptible to the bacteriophage activity. The susceptibility is increased if the bacteriophage is added to the bacterial culture after incubating for three hours. If the bacteriophage is added at the time of inoculation of the culture, the same increase in virulency is found during the beginning of the logarithmic growth, but the activity decreases on further growth of the bacteria. On the other hand, if the bacteriophage is added after 3 hours' incubation, the culture usually becomes sterile almost immediately and remains so for several days or weeks. At the end of this logarithmic or stimulation period there is another biological change in the bacteria in their relation to the bacteriophage. The change does not take place so rapidly as during the beginning of this period, nor does it last for so long a period of time. The greatest bacteriolytic activity is not manifested until 24 or 48 hours after the bacteriophage has been added to a 7 to 10 hour old bouillon culture of the susceptible microorganism. After a few days this passes off and no bacteriolytic activity can be demonstrated in such cultures.

We think that the constant results we have obtained in reference to the 3 hour old culture is due to the fact that we have been working with this particular strain *B. typhosus* (Olsen) for two years and the numerous transplants, etc., that have been made in our laboratory; using the same medium, temperature, etc., has accustomed this microorganism to the extent that its lag and logarithmic periods are almost constant. In a later paper dealing with bacterial counts this supposition will be substantiated by experimental evidence.

In this paper we wish to deal with this question of the logarithmic period of growth and its influence on bacteriophage activity, by means of using the Berkefeld filtrate of cultures during this and other phases of the life cycle of *B. typhosus*.

#### EXPERIMENTAL

The same technic was used in these experiments as was described in the preceding paper.<sup>5</sup> The fourth extraction of a bottom water-agar layer was used, the original bacteriophage was obtained from the feces of a typhoid

patient. Five flasks of bouillon, each containing 150 c.c. were incubated overnight and then inoculated with 1 c.c. of an 18 hour old bouillon culture of *B. typhosus* (Olsen). After 3 hours incubation the contents of one flask was passed through a Berkefeld filter; after 6, 9, 24, and 48 hours' incubation the contents of the other four flasks, respectively, were pulled through Berkefeld candles. The filtrates were adjusted, under sterile conditions, to a hydrogen-ion concentration of PH 7.0°, then pipetted into bouillon in the concentration of 1:1 and 1:4; two series were used.

The results of these experiments were negative. There is very little variation occurring in the various filtrate series as compared with the control bouillon.

In the first series of experiments made, we did not adjust the hydrogen-ion concentration, and as a consequence we obtained different results. We are now confident that these results were due to a variation in the reaction of the medium and not to the influence of any bi-products of metabolism present in the filtrates due to bacterial growth. Rahm<sup>2</sup> found that the 4, 6, 8, and 12 day old Berkefeld filtrates of *B. fluorescens liquefaciens* bouillon cultures did not exert much influence upon the life cycle of this microorganism. It is probable that if the change in reaction had been corrected, the small change he observed would not have occurred. Penfold<sup>3</sup> found the filtrate of a 17 hour old *B. coli* in bouillon did not effect the lag period of this bacteria. The changes that one observes in the reaction between a susceptible microorganism and a bacteriophage seems to depend upon the bacteria itself. It is not due to the medium in which the bacteria is growing at the time this change takes place.

The majority of the workers who have studied the lag and logarithmic periods of growth, have come to the conclusion that the cause for this must be within the bacteria itself. (Penfold, Chesney, etc.) We think the bacteriophage reacts best with the bacteria at certain periods of its life cycle, namely, the beginning and the end of the logarithmic growth period, this is independent of biproducts of growth of the bacteria present in the medium at this time.

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## SUGAR IN URINE\*

BY ROBERT L. THRASHER AND CHARLES W. O. BUNKER

BENEDICT'S qualitative test<sup>1</sup> for sugar in urine has proved very useful and reliable, and we have been curious as to whether results from its use have any quantitative significance. The method of Folin and Berglund<sup>2</sup> for the estimation of sugars in normal urine offered a simple means of deciding the question, and we have applied it to 190 urines, 109 of which were negative by Benedict's tests, while 81 showed greenish turbidities. Depending upon the density of this greenish turbidity, we have subdivided the group into traces, slight traces and very slight traces. Table I shows the results.

It will be noted that 88 per cent of the negative results were with urines containing 0.1 per cent or less of sugars. No sharp line, however, could be drawn as to the amount of sugars that would or would not give a positive result, although there was a tendency for the density of the greenish turbidity to vary, in general, proportionately to the amount present. Neither did we find that the occurrence of a reddish precipitate that settled to the bottom of the tube had any quantitative significance.

Folin and Berglund<sup>3</sup> contend that the copper-reducing substances remaining in normal urine after the removal of coloring matters, uric acid, creatine, and creatinine by means of the alkaloidal reagent consist of a "motley variety of carbohydrate products and carbohydrate derivatives including di- and polysaccharides." If we consider that Benedict's test is sensitive to some of these but not to others, we can understand why values as low as 0.04 per cent could produce either a positive or a negative result, depending upon the composition of the mixture present in the urine. It may also be that the relative proportions of fermentable to nonfermentable copper-reducing substances enter into the question, but we did not attack it from that standpoint.

Since a positive result was obtained with as little as 0.04 per cent of sugar, Benedict's qualitative test must be considered as having at least that sensitiveness.

### CONCLUSIONS

1. Benedict's qualitative test for sugar in urine has quantitative significance in only the most general terms.
2. Greenish turbidities secured in this test demand care in their interpretation, and are to be considered pathologic only when other features of the case warrant such an assumption.

\*From the Laboratories of the U. S. Naval Medical School.  
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TABLE I

MILLIGRAMS SUGAR PER 100 C.C. FOUND WITH THE FOLLOWING QUALITATIVE RESULTS

NEGATIVE	VERY SLIGHT TRACE	SLIGHT TRACE	TRACE
8			
10-11-11-13			
13			
20-22-23-23			
26-29			
31-32-33-35			
35-37-38			
40-40-40-40			
40-43-43		40-42	
43-43-46			
46-46-46			
47			
50-50-50-50			
52-53-53			
54-54-54			
54-57-57			
57			
60-60-60-60			
60-60-60		66	
63-63-63			
66-66-66			
66-66-66			
67			
70-71-71-72			
72-73-75		75-75	71
80-80-80-80	80	70-70-75-75	
80-80-80		80-80-80-85	80-80-85
85-85-85		85-86-86	
86-86-86			
86			
92-92-92	91-95	90-92-92-95	
100-100-100		95-95	
100-100		100-100-109	104-109-109
100-100		109-109	
109-109		109	
110-110-114			
114			110-111-114
120-120-120			114
120		120	120-120-120
133-133-133		133-133-136	128-128
			133-133
150	150	150-150-150	142
			150-150-150
			150 155
			163
			171-171-171
			171-177
			180-180-180
			188
		190	
			200-200
		218	
		240	
			224
			257-257
			500

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## LABORATORY METHODS

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### ULTRAFILTRATION OF BLOOD SERUM AND CEREBROSPINAL FLUID WITH SPECIAL REFERENCE TO A NEW APPARATUS\*

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BY GRETE EGERER-SEHAM, PH.D., MINNEAPOLIS, MINN.

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SINCE Quincke, in 1891, brought the spinal puncture into clinical use the studies on the cerebrospinal fluid have greatly increased, especially since the introduction of accurate and sensitive microchemical and biological reactions. Notwithstanding, our knowledge of the physiology and pathology of the cerebrospinal fluid is still relatively incomplete.

It is commonly accepted that the fluid is derived from the blood and that its source is the chorioid plexus. How the fluid is formed and what rôle the chorioid plexus plays in its formation is, as yet, a matter of speculation. The exact nature of cerebrospinal fluid, i.e., whether it is an active secretion or a filtrate has never been determined.

McClendon has suggested that the cerebrospinal fluid is an ultrafiltrate of blood serum. He showed that a fluid could be produced by ultrafiltration of serum that resembled, in at least several physical and chemical characteristics, the cerebrospinal fluid. His work seemed to open a field of investigation that might prove fruitful.

This paper is concerned with the ultrafiltration of blood serum, special attention being given to the presence or absence in the ultrafiltrate of globulin, the colloidal gold curve and the Wassermann reaction.

The first problem was to devise an apparatus and method of ultrafiltration that would be as simple as possible and yet give accurate and quantitative results. The apparatus finally made and used is the one shown in Fig. 1. It is similar in principle to the one devised by Bechold, but much simpler in construction, rendering ultrafiltration comparatively easy.

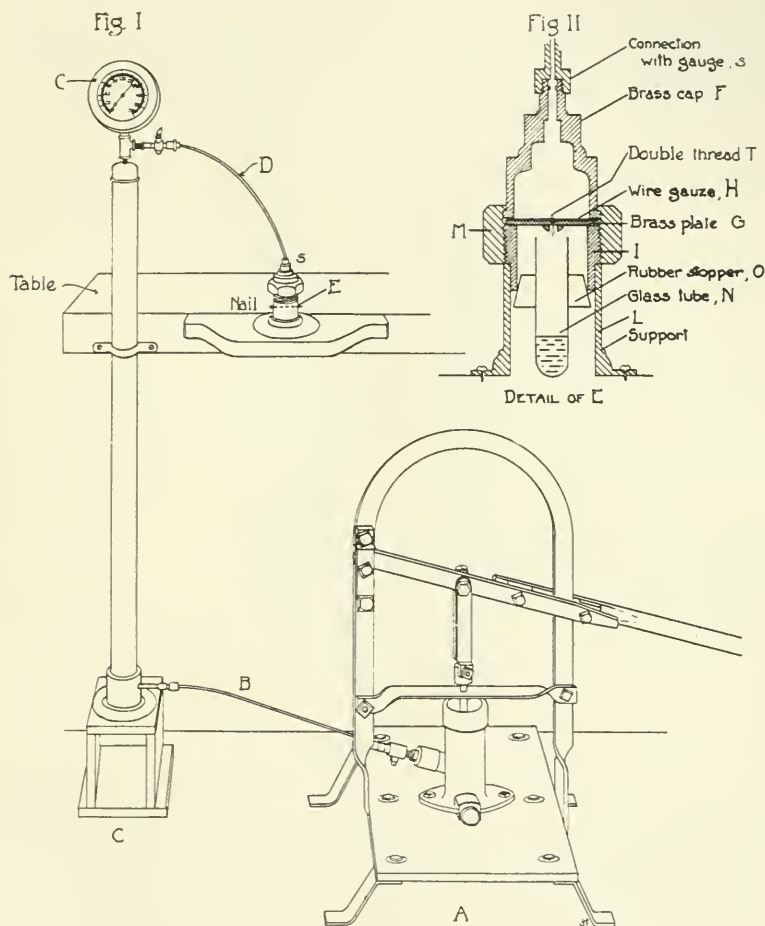
The ultrafiltration apparatus described in this paper has the following advantages: (1) It allows as complete a quantitative ultrafiltration as can be expected. (2) The apparatus is easily constructed at a small expense. (3) The experiments can be carried out with great ease in relatively short time.

Fig. 1 shows the apparatus ready for use and Fig. 2 represents a cross section of the apparatus. It consists of an ordinary air pump (*A*) which is connected by means of a metal tube (*B*) to a gauge (*C*) from which a brass tube (*D*) leads to the apparatus proper (*E*). This apparatus (*E*) consists

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of an upper cap (*F*) made from brass tubing. This rests on a brass plate (*G*)  $1\frac{1}{2}$  inches in diameter, perforated in the center, a wire gauze of very fine mesh rests upon the brass plate occupying about  $\frac{4}{7}$  of the entire circumference. The edge of the gauze is on the same level with the rim of the brass plate. A double thread (*I*) taken from the same wire gauze is passed through the center of the gauze and through the perforation in the plate (*G*). The plate lies on the receptacle (*I*) made of brass tubing with two holes in juxtaposition.



Figs. 1 and 2.

position. In order to screw together the cap (*F*) and the receptacle, a heavy metal nail is pushed through both lower receptacles (*I* and *L*). In order to avoid contamination of the fluid examined, certain parts of the apparatus (*F-H-I-G*) are coated with gold. Experiments are carried out in the following way:

Receptacle *L* is fastened to the table. It is sufficiently large to hold the receptacle *I* and it is perforated on either side like receptacle *I*. Part *I* is placed into the receptacle *L* and the nail is passed through the holes. The

filter plate *G* is placed upon the receptacle *I* and the wire gauze *H* is put over the plate with the wire thread passing through the pin hole. The filter paper  $1\frac{1}{2}$  inches wide, coated with collodion, is put on the wire gauze. A rubber washer of the same diameter as the brass plate is used. Lastly part *F* is placed on the rubber ring and the screw cap *M* that holds the parts together adjusted airtight with a wrench. A small glass tube (*N*) fitted with a bored rubber stopper (*O*) is employed for catching the ultrafiltrate. The fluid to be ultrafiltered is introduced into receptacle *F* by means of a pipette fitted with an about 3-mm. wide rubber tubing. The apparatus is screwed on by means of the screw cap (*s*) and tightened with a small wrench. Air is pumped into the apparatus until the gauge registers the desired pressure which is expressed in pounds.

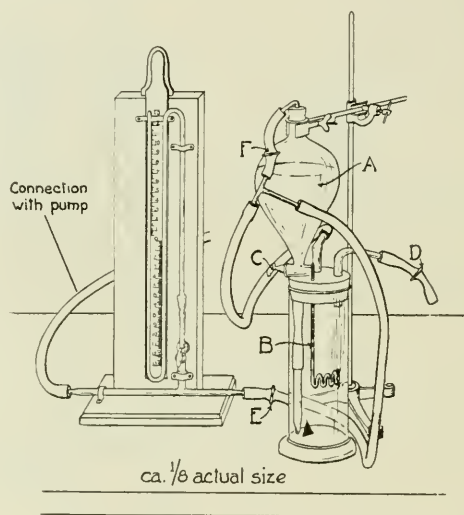


Fig. 3.

#### COATING OF THE FILTER DISCS

Two kinds of apparatus were used in coating the filter discs.\* The apparatus shown in Fig. 3 can be employed when but one or two filter discs are needed for ultrafiltration, whereas the apparatus represented by Fig. 4 permits the coating of nine filter discs at a time.

#### DESCRIPTION OF APPARATUS SHOWN IN FIGURE 3

The apparatus consists of a suction pump, a manometer, a glass cylinder fitted with a four-hole stopper, and a separatory funnel with a one-hole rubber stopper. The funnel is filled with the collodion solution† and the filter disc is sewed to the glass rod by means of cotton thread. The screw-cock at *D* is tightened while the screw-cocks at *E* and *F* are loosened. Then the apparatus is evacuated. When it is air free the pinch-cock *F* is closed and the

\*The filter discs are cut from strong Japanese filter paper.

†Anthony's negative cotton, dried on an electric plate at 80° is dissolved in glacial acetic acid, that contains 2-1½ per cent sodium carbonate.

stop-cock *C* is slightly opened to permit a slow flow of the collodion solution into the cylinder. When the filter disc is completely submerged in the solution stopcock *C* is closed again. Suction is continued until there cannot be seen any air bubbles in the solution. Then the cylinder and the suction pump are disconnected (screwcock *E* is closed). The screwcock *D* and stop-cock *C* are opened. Air thus admitted drives the solution from the cylinder back into the separatory funnel. The rubber stopper carrying the rod with

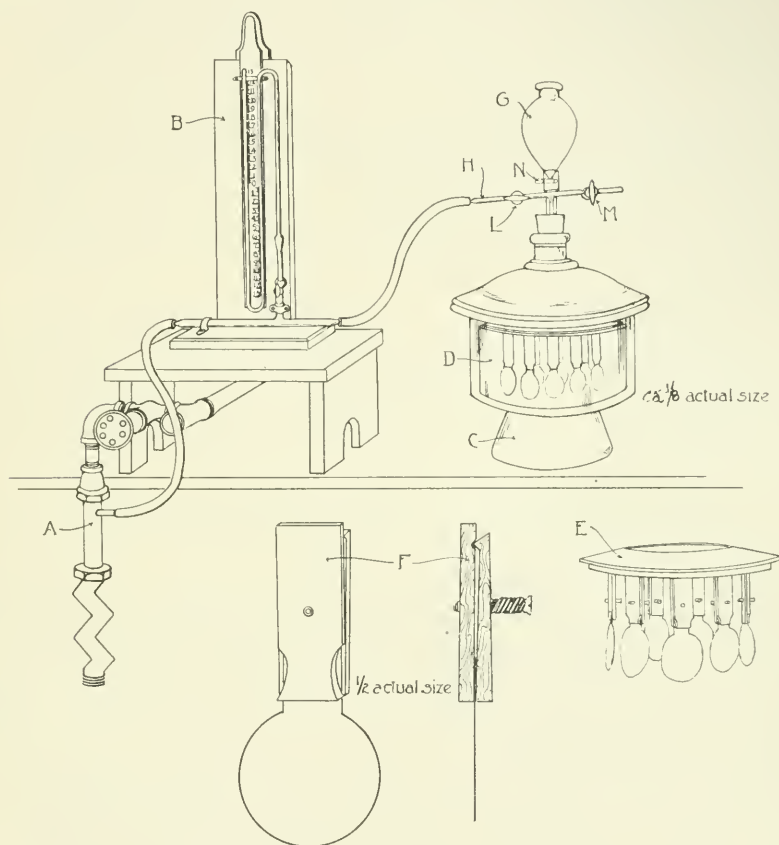


Fig. 4.

the filter disc is lifted out of the cylinder. Ten seconds are allowed between two drops of the excess solution on the disc before the disc is submerged in running water. The filter disc is kept in running water for two days. It is then preserved in distilled water with a drop of chloroform until further use.

#### DESCRIPTION OF APPARATUS SHOWN IN FIGURE 4

The apparatus consists of a strong suction pump (*A*), a manometer (*B*), a desiccator (*C*), into which a glass jar (*D*) is fitted. This glass jar carried a wooden ring (*E*) to which nine clamps (*F*) are glued. The clamps are made of two strips of wood held together by a simple spiral-shaped spring. The



rubber stopper carries a funnel (*G*). A U tube (*H*) with two stopcocks connects the apparatus with the manometer on one side and with the air supply on the other side.

After the apparatus has been evacuated (stopcock *L* open, stopcocks *M* and *N* closed) part of the solution is admitted into the desiccator through the stopcock *N*. When the manometer again registers evacuation more fluid is admitted, and so on until the filter discs are completely covered. Care must be taken that the collodion solution does not reach the spiral-shaped springs. When the solution is free from air bubbles the suction is discontinued and air is admitted through stopcock *M*. For the completion of the experiment see the above directions.

Before entering into a discussion of the ultrafiltration of blood serum a short description of Lange's colloidal gold test may be in place. The reading of the colloidal gold test depends on various color changes in the tubes of different dilutions.

With normal spinal fluid there is no change of the red color in any of the ten tubes.

In pathologic fluids there may be color changes ranging from a bluish tinge to a red, to complete decolorization, and these changes have been designated by numbers, the unchanged color being designated as zero and complete precipitation by No. 5, the intermediary colors being the numbers between zero and 5.

The clinical application of the colloidal gold test depends on varying degrees of reaction, according to the dilution of the spinal fluid. In general, three types of reaction are found:

1. Those in which the color changes, which depend on the precipitation of the colloidal gold, occur in the first type. This type of reaction has been given the name paretic curve and such a curve would mean 5, 5, 5, 5, 4, 2, 1, 0, 0, 0.

2. Type of curve is one occurring in the middle tubes or in the second zone, giving the curve such as 0, 0, 0, 1, 3, 4, 3, 2, 0, 0. This type of curve is quickly found in tabes dorsalis.

3. Type of curve is known as the meningitic curve and is found in those cases where the precipitation occurs in the tubes of highest dilution or in the third zone. Type of meningitic curve would be 0, 0, 0, 0, 1, 4, 5, 5, 4, 1.

The curves occurring in zones 1, 2, and 3, are not specific for paresis, cerebrospinal syphilis or meningitis. Rare other diseases of the central nervous system may give similar curves.

#### ULTRAFILTRATION OF NORMAL BLOOD SERUM

The blood was collected in a thoroughly cleansed test tube, allowed to stand in the ice box for not more than half an hour and then centrifuged for 15 minutes. Of the separated serum 3 c.c. were used for the experiments and the globulin test and colloidal gold curve carried out in the second c.c. of the ultrafiltrate, the first c.c. of the ultrafiltrate being discarded. Filter discs coated with 3.5 per cent, 4.0 per cent and 4.5 per cent collodion solution were employed. The results are given in Table I.

TABLE I.

CONC. OF COATING	GLOBULIN TEST	COLLOIDAL GOLD CURVE
3.5 per cent	Negative	0002100000
4. " "	"	0121100000
4. " "	"	0112200000
4. " "	"	0002000000
4. " "	"	1112311000
4. " "	"	3110000000
4.5 " "	Trace	0000132200
4.5 " "	Trace	0000131100*
4.5 " "	Negative	3333221000*

\*Hemolyzed serum.

In all cases the nitric acid test was strongly positive, and in all but two cases the ammonium sulphate test (globulin test) negative. All ultrafiltrates gave a weak reduction of the colloidal gold solution. To show that the colloidal gold curve in the ultrafiltrate is rather independent of the colloidal gold curve in the blood serum the following two cases may be cited:

	SERUM	ULTRAFILTRATE 4 PER CENT
Colloidal gold curve	5555555554	1112311000
	0004555555	3110000000

All ultrafiltration even of hemolyzed serum yielded colorless ultrafiltrates. Ultrafiltration of normal blood serum shows the presence of a substance that reduces colloidal gold solution.

## ULTRAFILTRATION OF SYPHILITIC CEREBROSPINAL FLUID

Spinal fluids were ultrafiltered through discs coated with 3.5 per cent, 4.0 per cent, 4.5 per cent collodion solution. Ultrafiltrates of normal spinal fluids gave no colloidal gold curve. The reduction of colloidal gold solution by ultrafiltrates from syphilitic spinal fluids seems to depend on the quantity of substance present in the spinal fluid reducing the colloidal gold solution. If for instance spinal fluid yielding a weak reduction curve was ultrafiltered the colloidal gold curve of the ultrafiltrate was practically negative. If on the other hand spinal fluid that gave a very strong colloidal gold curve was ultrafiltered the filtrate exhibited a positive colloidal gold curve. This was best shown in the following case:

CONC. OF COATING	SPINAL FLUID	ULTRAFILTRATE
3.5 per cent	55553321000	5554110000

## GENERAL REACTIONS IN ULTRAFILTRATES

Sera with positive Wassermann reactions were ultrafiltered through paper coated with 4.0 and 3.5 per cent collodion glacial acetic acid solution. The results are tabulated in Table II.

Of the two sera ultrafiltered through 4 per cent coating both had a definite colloidal gold curve and positive globulin tests. The same sera when ultrafiltered through 3.5 per cent coating gave no colloidal gold curve. Only one serum ultrafiltered through 3.5 per cent coating gave a colloidal gold curve.

TABLE II

NUMBER	SERUM			U SERUM 4%			U SERUM 3-1/2%			REMARKS
	W.	C.G.	GL.	W.	C.G.	GL.	W.	C.G.	GL.	
0861	4 pos.	32s3		?	w3	2 pos.	1 pos.	neg.	?	control to U3-1/2 ant.
115764B	4 pos.	s1s2s3		neg.	w2w3	2 pos.	?	neg.		
115055J	4 pos.	neg.					neg.	w3		
2255R	1 pos.	w2w3	2 pos.				w3	neg.		
17686R	?	s1s2	2 pos.		w2			neg.		
17749D	?	neg.			w1w2	?	w2w3	trace		
17686	neg.	n-g.	4 pos.	neg.	neg.	1/2 pos.	neg.	neg.	neg.	
175770	neg.			neg.			neg.			
H	neg.	w3	2 pos.				neg.	2 pos.		

*Keys*

W = Wassermann reaction.

c. g. = colloidal gold test.

U serum = Ultrafiltrate of serum

U serum 4% = serum ultrafiltered through filter paper coated with 4% collodion acetic acid solution.

U serum 3 1/2% = serum ultrafiltered through paper coated with 3 1/2% collodion acetic acid solution.

s2s3 = strong reduction in tubes 456, and tubes 789.

w2w3 = weak reduction in tubes 456, and tubes 789.

4 pos. = four-plus signs.

Of two bloods with a questionable Wassermann one gave a colloidal curve in the serum ultrafiltered through filter paper coated with a 4 per cent solution and the other ultrafiltered through a disc coated with a 3.5 per cent solution.

Ultrafiltration of nonluetic bloods (negative Wassermann) was run as controls. The ultrafiltrate did not give a colloidal gold curve; two of them, however, had a positive globulin test.

## CONCLUSIONS

1. The ammonium sulphate test (globulin test) is negative in the majority of ultrafiltrates of normal blood serum.

2. The ultrafiltrates of nonsyphilitic blood sera give a reduction of the colloidal gold solution in almost all cases. The same is true of the ultrafiltrates of syphilitic sera.

3. The ultrafiltrates of normal cerebrospinal fluid give no colloidal gold curve.

4. The reduction of colloidal gold solution by ultrafiltrates from syphilitic spinal fluids seems to vary with the quantity of the substance reducing the colloidal gold solution present in the original spinal fluid.

5. The substance giving the Wassermann reaction in syphilitic blood does not seem to be ultrafiltrable.

NOTE: I wish to express my appreciation to Mr. Victor Roehrich, St. Paul, for making the ultrafiltration apparatus, to Dr. Margaret Warwick for the determinations of the colloidal gold tests, and to Dr. H. T. Ogden for the determination of the Wassermann reaction.

## METHOD AND DIAGNOSTIC VALUE OF QUANTITATIVE DETERMINATION OF SUGAR IN THE CEREBROSPINAL FLUID\*

BY GUY H. MOATES, M.D., AND J. JAY KEEGAN, M.D., OMAHA, NEBR.

THE quantitative determination of sugar in the cerebrospinal fluid has been a rather recent development in laboratory diagnosis.<sup>3</sup> It has received special attention in relation to epidemic encephalitis in which it has been claimed by several, Thalimer and Updegraff,<sup>4</sup> Foster,<sup>5</sup> Netter, Bloch, and Dekemwer,<sup>6</sup> Kraus and Pardee<sup>7</sup> and Coope.<sup>8</sup> to be fairly constantly increased.

Several methods have been advocated in the last two or three years and apparently the most accurate results have been obtained by some modification of the technic used by Lewis and Benedict<sup>2</sup> for the quantitative determination of sugar in small amounts of blood. The method used in this series is a modification of this technic using 4.0 c.c. of cerebrospinal fluid instead of blood. This method seems to have the advantage over any other because there is a standard color which is prepared from a solution with a known glucose content and also one is comparing shades of red and yellow which can be matched with a lesser degree of error than the blue of copper sulphate solutions.

The rapid methylene blue method advocated by Yasharia and Hattori<sup>1</sup> was used to compare some of the readings in this work, but it was abandoned as a routine measure because it was so unreliable and no accurate idea of the amount of sugar present could be obtained.

There has been considerable discussion as to the percentage where the sugar content of the cerebrospinal fluid is to be considered pathological or if it has any diagnostic value. Two hundred three specimens were examined in this series. No attempt was made to select them from any particular group of patients, but they were taken from specimens that were sent to the laboratory for routine examination.

### CEREBROSPINAL SUGAR

Table I shows that the average sugar content for the 203 specimens was 0.0585 per cent. The highest reading was obtained in a severe case of epidemic encephalitis, being 0.288 per cent, and the lowest reading was in a case of tuberculous meningitis, being 0.013 per cent. The highest average for any series of cases was in the group of epidemic encephalitis, being 0.081 per cent, and the lowest readings were obtained in the group of meningitis of infectious character. These findings are practically in accordance with those of Thalimer and Updegraff;<sup>4</sup> Foster;<sup>5</sup> Netter, Bloch, and Dekemwer;<sup>6</sup> Kraus and Pardee;<sup>7</sup> and Coope.<sup>8</sup>

\*From the Department of Pathology, University of Nebraska, College of Medicine, Omaha.



A series of experiments were performed on a large dog to determine the effects of various substances on the sugar content of the cerebrospinal fluid obtained by eisternal puncture. A normal fluid was first obtained and found to contain 0.086 per cent sugar. Two-tenths of a cubic centimeter of turpentine in one cubic centimeter of physiological sodium chloride was injected into the eisternal space. There was a definite increase in the cell count to 105 per cubic millimeter and a slight increase in the sugar content to 0.092

TABLE I

SHOWING THE AVERAGE, THE HIGHEST AND THE LOWEST, PERCENTAGE READINGS OF THE SUGAR CONTENT OF THE CEREBROSPINAL FLUID IN DIFFERENT DISEASES

CLINICAL DIAGNOSIS	NUMBER	AVERAGE %	HIGH %	LOW %
Tabes dorsalis	35.	0.055	0.075	0.031
Syphilis, not of the central nervous system	33	0.054	0.101	0.041
Paresis	32	0.059	0.090	0.045
Epidemic encephalitis	29	0.081	0.288	0.046
Tabes-parcisis	8	0.054	0.069	0.046
Dementia praecox	6	0.047	0.054	0.040
Syphilitic optic atrophy	6	0.053	0.070	0.038
Toxic psychoses	5	0.055	0.065	0.051
Vascular C-S syphilis	5	0.063	0.085	0.046
Psycho-neuroses	4	0.055	0.065	0.040
Tuberculous meningitis	4	0.0175	0.021	0.013
Alcoholic psychoses	4	0.050	0.063	0.041
Arteriosclerosis dementia	4	0.058	0.060	0.056
Syphilitic meningitis	3	0.063	0.120	0.031
Paralysis agitans	3	0.076	0.088	0.066
Typhoid meningitis	3	0.030	0.032	0.028
Nephritis	2	0.049	0.055	0.042
Manic-depressive psychoses	2	0.062	0.062	0.062
Epilepsy	2	0.065	0.065	0.065
Meningococcus meningitis	2	0.020	0.032	0.018
Alcoholic meningismus	1	0.107	0.107	0.107
Toxic meningismus	1	0.096	0.096	0.096
Miliary tuberculosis	1	0.062	0.062	0.062
Brain tumor	1	0.042	0.042	0.042
Spinal cord tumor	1	0.050	0.050	0.050
Cerebellar hemorrhage	1	0.117	0.117	0.117
Post traumatic syndrome	1	0.091	0.091	0.091
Pneumococcus meningitis	1	0.025	0.025	0.025
Sinusitis	1	0.048	0.048	0.048
Normal individual	1	0.050	0.050	0.050
Total	203	0.0585	.....	.....

per cent in the fluid which was withdrawn 48 hours later. The dog was allowed to rest for 6 days and another puncture was performed and normal fluid obtained. At this time 2 drops of Dakin's hypochlorite solution were injected with a resultant immediate severe reaction. Fluid was obtained the next day and showed a cell count of 4,150, increased protein, but a normal sugar content, 0.083 per cent. Fluid was obtained on each of the three following days with no significant difference in the sugar content and with a steady decrease in the cell count and the protein. After the fluid had become normal, the dog was placed under ether anesthesia and fluid was obtained. This showed a normal cell count and protein, but a slight increase in the sugar content, 0.098 per cent. This increase might be attributed to the stimulating effect of ether anesthesia on the secretion of the choroid plexus.<sup>14</sup>

## DISCUSSION

An analysis of the 203 specimens studied shows a definite increase in the sugar content in most of the cases associated with a cerebral irritation. The other cases are within the limits which were noted by Foster<sup>5</sup> as being a normal sugar content of the cerebrospinal fluid. These findings disagree with those of Stevenson<sup>9</sup> who found no significant increase of sugar in the cases of epidemic encephalitis and a greater variation in the cases of neurosyphilis.

Several authors have attempted to show some connection between the colloidal gold curve, the cerebrospinal fluid Wassermann, the cell count, the protein and the sugar content. In this series there is no evident relationship between the sugar content and any of the other serological findings. No case of epidemic encephalitis showed a typical paretic curve as reported by Howell,<sup>10</sup> there being no characteristic colloidal gold curve observed.

The cause of hyperglycorrhachia is as yet an unsolved problem. Kahler<sup>11</sup> thinks it is caused by most stimulations and inflammations of the brain. Hypoglycorrhachia in the cases of meningitis is probably due to the reducing action of the invading microorganism and not due to the inflammation they cause. Specimens of cerebrospinal fluid which were collected under sterile precautions and kept in the ice box (8° C.) did not vary any appreciable amount in sugar content and could be used for quantitative determinations 96 hours after having been withdrawn. These same specimens when inoculated with the pneumococcus, certain strains of the streptococci, the meningococcus or the tubercle Bacillus and kept at ice box temperature 24 hours showed a constant decrease in the sugar content.

It has been shown fairly definitely by several investigators<sup>15</sup> that certain strains of the pleomorphic streptococci have no reducing powers. Such a microorganism producing inflammation of the brain, as is suspected in epidemic encephalitis, might be the cause of hyperglycorrhachia. This would be an additional point in favor of Bonhoeffer's<sup>12</sup> claim that encephalitis is due to a pleomorphic streptococcus. Hassin<sup>13</sup> states that epidemic encephalitis is an inflammation and not a degenerative process and this statement agrees with that of Kahler<sup>11</sup> and that the hyperglycorrhachia is due to inflammation and overstimulation. This may explain the high sugar content in the cases of alcoholic and toxic meningismus of this series.

Thalmer and Updegraff<sup>4</sup> think the high sugar content of the cerebrospinal fluid is due to a change in the blood sugar, but this does not seem possible as none of the cases of encephalitis of this series showed a glycosuria or a glycemia.

Kahler<sup>11</sup> gives three tentative explanations of the hyperglycorrhachia: (a) abnormal permeability of the choroid plexus for sugar, based on Blumethal's work showing that the cerebrospinal fluid is formed by transudation and secretion; (b) abnormal secretion of glucose in the plexus cells based in the findings of Yoshimura who demonstrated glycogen in these cells and (c) abnormal metabolic process in the brain substance. The last two are the most commonly accepted at present.

## CONCLUSIONS

1. The normal range of the cerebrospinal sugar is between 0.040 per cent and 0.068 per cent.
2. Sugar content of the cerebrospinal fluid is distinctly increased in most cases of epidemic encephalitis and has a diagnostic significance.
3. Sugar content of the cerebrospinal fluid is decreased in infectious meningitis probably due to the reducing powers of the invading microorganisms.
4. There is no apparent relation between the sugar content of the cerebrospinal fluid and the Wassermann reaction, colloidal gold curve, cell count, globulin or total protein.

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## EDITORIALS

### *Sodium Chlorid Restriction in Hypertension*

THE treatment of hypertension with diets of low salt content is not new. Such dietary measures have been studied in the past chiefly by various French observers. In this country, as a rule, salt restriction has not been pushed to an extreme, and only occasional investigators have insisted upon a chlorid intake of less than two grams per day. Opinions of the efficacy of rigid salt restriction have been distinctly at variance. This appears due, in part at least, to the fact that salt restriction has not often been enforced rigidly enough to produce the desired results. Allen and Sherrill, who have given much time to a study of this method, find that best results are obtained with a chlorid intake sufficiently reduced that no more than one-half gram of sodium chlorid is excreted in the twenty-four hour urine. Since this rigid restriction occasionally causes deficiency symptoms, they insist that no clinician who has used salt restriction with unsatisfactory results, has used it properly unless he has observed salt deficiency symptoms.

The Morristown investigators have worked on the hypothesis that hypertension, particularly uncomplicated hypertension, is a disease resulting from



error in salt metabolism, and that individuals with such symptoms are unable to tolerate normal quantities of sodium chlorid. Their results tend to substantiate this claim. As yet they make no suggestion as to the underlying cause for the error in salt metabolism.

Normal blood pressure was restored in 18.9 per cent of 180 severe cases, therapeutic success without complete restoration of normal pressure was obtained in 41.9 per cent, and transitory benefit followed by relapse or death occurred in 8.9 per cent. Complete failure was encountered in 30.5 per cent. Treatment lasted over periods from one month to one year. The entire group was divided into four classes. Those with pure hypertension and high plasma chlorid values, those with uncomplicated hypertension and high plasma chlorid values, those with uncomplicated hypertension and relatively low blood chlorids, cases with associated nephritis, and those with diabetes. Best results were observed in the first group. A suggested explanation of less successful results in the second group is that in the first the hypertension was more purely functional and associated with the high chlorid readings, while in the second the increased pressure followed more permanent organic changes. Poorest results were observed in those cases associated with nephritis. Complicating diabetes, when properly treated, in no way interfered with satisfactory results from salt restriction. Although uncomplicated cases were classified according to high and low plasma chlorid content, the authors found no constant correlation in their series between the chlorid level and the degree of hypertension. The authors devote little attention to a classification of their cases according to the degree of arteriosclerosis present. We hope that this phase will receive further study.

Allen and Sherrill found no such close association between obesity and hypertension as was formerly assumed to hold. The majority of patients had normal or slightly subnormal body weights. Improvement occurred independently of the presence or absence of abnormal weight, except when increased weight was due to fluid retention with or without recognizable edema. They found that a normal blood urea nitrogen concentration on a liberal protein diet is probably as conclusive indication of adequate nitrogen excretion as any other single test. Retention of urea or nonprotein nitrogen was found to bear no relationship whatever to the degree of hypertension. In those cases with impaired renal function and nitrogenous retention, protein intake was restricted along with salt intake, but with normal nitrogenous metabolism the protein intake was not reduced at all. In some of the more severe nephritides it was found necessary to first treat the nephritis with a low protein diet and later to reduce the salt intake. Otherwise alarming uremic symptoms occasionally developed. The latter were quickly relieved by the giving of ten grams of sodium chlorid daily. Albuminuria and cylindruria were usually uninfluenced by salt restriction. The minority of cases showed unmistakable diminution thereof.

Increased plasma sugar values have recently been described in essential hypertension and improvement in symptoms has been ascribed to reduction of carbohydrate intake. Allen and Sherrill found the blood sugar values

normal in a higher percentage of group 1 cases than in the second group with low plasma chlorid levels. In the third group, associated with nephritis, the plasma sugar was still more constantly elevated. It is suggested that the increased sugar values occur at a later stage of the disease, when permanent organic changes have occurred and may be due to arteriosclerotic changes in the pancreas.

In cases successfully treated there was a fall not only of the systolic but also of the diastolic blood pressure. The latter was distinctly more stubborn and was reduced to a less extent than the former. It is a matter of general observation that hypertension cases in which the blood pressure has been reduced to too great an extent are subjectively and often objectively much more seriously affected than when the pressure was higher. For this reason certain authors have assumed that the development of hypertension is primarily a compensatory affair and attempts should not be made to alter it to too great a degree. When the blood pressure has fallen with resulting unpleasant symptoms, it is usually found that the systolic pressure has been decreased out of proportion to the diastolic reduction and that the pulse pressure has thereby become unusually small. It appears probable that the unpleasant symptoms have been due to the resulting small pulse pressure, with correspondingly reduced functional capacity of the cardiovascular apparatus. If strict salt restriction will reduce the diastolic pressure proportionately or nearly proportionately to the systolic reduction, we will be in a position to understand and to expect good clinical results from the method advocated by Allen and Sherrill.

The results in cases treated by Allen and Sherrill were obtained after treatment varying from a few days to as long as one year. This indicates the necessity of perseverance in the diet even though immediate improvement is not observed. Those cases responding promptly with fall in blood pressure are of better prognosis than those in which the blood pressure is reduced only after a longer period. The diet was regarded as "salt-free" when the sodium chlorid content of the urine was kept at or below 0.5 gm. per day. Most of the individuals remained on such a diet continuously. Occasionally privation symptoms occurred with weakness, anorexia, vertigo, etc., necessitating an increase in the salt intake. A careful balance must finally be attained, the patient being given a sufficiently low salt intake that he may show no salt retention symptoms and yet sufficiently high that privation symptoms do not supervene. Since the levels at which both groups of symptoms appear vary in individual cases, this sometimes requires patient standardization. The most difficult cases are those in which the pressure is only slightly reduced by a chlorid restriction which causes privation symptoms. The best clinical diet in these hopeless cases is afforded by a chlorid allowance which gives a mixture of mild privation symptoms and mitigated hypertension symptoms.

Chlorid restriction was found to act favorably on the serious accompaniments of hypertension, such as cardiac failure, incipient pulmonary edema, retinitis and vascular crises, including to some extent angina pectoris.

The authors emphasize that their work has been done on a series of severe cases and that the probability of improvement would be correspondingly greater in a series with mild or early hypertension. No attempt is made to explain the presence of an abnormal salt metabolism predisposing to hypertension. It is the experience of most of us that individuals with hypertension are unusually heavy salt eaters and it may be that the disease may arise in part from chronic overload.

The actual limitations of our knowledge of the etiology of hypertension are evidenced by the multiplicity of therapeutic procedures. The majority, such as hydrotherapy, bleeding, vasodilation, rest, etc., are purely symptomatic. It has long been supposed that hypertension is in some way related to error in protein metabolism, and protein restriction has therefore been the most generally accepted treatment. Just as typhoid fever and the paratyphoids were formerly classed under the general diagnosis of typhus fever, so hypertension was formerly associated exclusively with chronic nephritis and considered to be an event of the later stages of Bright's disease. With the recognition of essential hypertension as a distinct disease which may occur with minimal renal lesions a distinct diagnostic advance was made, but the treatment has continued to be based upon the older concept of protein restriction in renal disease. We can scarcely yet state that an error in protein metabolism is not the cause of essential hypertension. It does appear, however, that nitrogenous metabolism varies around normal limits in this disease and that the feeding of relatively high protein diets in no way interfered with good results from salt restriction. Most experimental work with high protein diets has been directed to the production of arteriosclerosis rather than hypertension. Thus Newburgh has recently produced arteriosclerosis strictly by high protein feeding. However, the production of such a condition experimentally in an herbivorous animal is quite dissimilar to the same procedure in man.

Strause and Kelman fed hypertensive individuals with and without associated nephritis, varying daily protein intakes. They could discover no relationship between the protein intake and the blood pressure. Nitrogen retention was influenced by changes in nitrogen intake but the blood pressure could not be so influenced. Squier and Newburgh, after feeding patients with hypertension amounts of protein well over 150 gms. per day, concluded that high protein diets over a short period have no effect on the blood pressure.

The occurrence of hyperglycemia in hypertension, emphasized especially by Herrick, may be explained merely as a secondary phenomenon resulting from organic changes in the pancreas, or, in cases associated with nephritis, as analogous to the nitrogenous retention.

It should be noted that the findings of Allen and Sherrill with regard to hyperglycemia are somewhat at variance with those recorded by Härle. Whereas the former found the plasma sugar value most constantly elevated in hypertensive cases with nephritis, Härle found the reverse to be the case. In one-half of cases with kidney lesions and hypertension the blood sugar was

on the upper border of the normal range. Frank hyperglycemia was rarely observed. On the other hand, in cases without demonstrable renal lesions, a definite hyperglycemia was seen in one-fourth and an upper normal range in another one-fourth. Botti found blood sugar increased and sugar tolerance diminished in the presence of hypertension, especially when associated with arteriosclerosis.

Following the work of Cannon, many have suggested that hypertension results from an increased adrenalin discharge in the system. Hülse was unable to demonstrate increased adrenalin concentration in the arterial or venous blood of hypertensive individuals, even though his frog preparation was sensitive to concentrations of adrenalin of one to one billion.

Orr and Innes, working on the hypothesis that pressor substances are derived from protein split products, regulated the fluid intake of their patients, and found that with increased fluid consumption the blood pressure was somewhat reduced. Their interpretation was that the hypothetical pressor substances were diluted, and perhaps to some extent removed by the increased diuresis. Their variations were not, however, unquestionably beyond the normal range.

Among other therapeutic measures based upon conceptions of the etiology of hypertension we must still number prohibition of nicotine and alcohol, reduction of overweight and removal of focal infection. The purin bases have ceased to incite terror.

One cannot say that it has been shown that hypertension is due to an error in salt metabolism. Nevertheless it is worthy of note that Allen and Sherrill have successfully relieved a fairly large proportion of their severe hypertensive cases by rigid restriction of salt intake without the application of other therapeutic measures.

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—W. T. V.

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#### *At Last, the Bed Bug*

THIS occasional intruder has until now held a position of inferior importance among pcutaneous feeders in that it has been considered to be nosetiologically innocuous. L. H. Dunn, entomologist at the Board of Health Laboratory, Ancon, Canal Zone, in 1921, conducted several experiments to determine any relationship between the tropical bed bug and the transmission of relapsing fever. Although unable to complete his work before his departure from Panama, Dr. Dunn made several interesting and suggestive observations. He first attempted to transmit the disease through the bite of



the bug. Thus he allowed 49 hungry bugs to bite an individual ill with relapsing fever, and on each of the seven succeeding days each bug fed upon a single white rat. During this period the rat received 704 bites. He did not develop relapsing fever. In the same way bugs were fed on infected rats and later were allowed to bite monkeys. The monkeys did not develop relapsing fever. It was demonstrated, however, that at the time of feeding on the monkeys the bugs were carrying spirochetes in the intestinal contents and in the salivary glands.

Positive results were obtained by mouse inoculation with suspensions of macerated bugs which had been allowed to feed on infected animals as long as thirty days previously. On dissection, bugs were found to be infected with spirochetes two days, eight days and fifteen days after feeding on infected animals. The microorganisms were present in the gut contents and salivary glands two days after feeding but were absent from these localities eight and fifteen days after. At these two later intervals spirilla were found in the coelomic fluid. Not all bugs were infected.

Although bed bugs did not experimentally transmit relapsing fever the observation that they are infected for appreciable periods after feeding on hosts sick with the disease is of considerable interest. The fact that relapsing fever was caused by the injection of macerated specimens of such bugs calls for further work along this line. More detailed control studies will be necessary. It is unfortunate that the author was unable to complete his studies before leaving the canal zone.

—W. T. V.

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\*Dunn: *Am. J. Trop. Med.*, 1923, iii, 315.

## BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

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### *The Urethra and the Urethroscope\**

THE author's experience has eminently fitted him to conceive and write this small handbook. He describes the anatomy and surface appearance of the normal male and female urethra as seen through the urethroscope, together with the appearance in various pathological conditions. He discusses the comparative virtues of different types of urethroscopes and outlines the accepted methods of treatment without discussing in detail moot therapeutic considerations. Though condensed, the book is quite thorough and should be highly useful to those doing urethroscopic work. It is well illustrated.

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### *Practical Dietetics†*

WITH the rapidly changing and developing concepts of dietary treatment in various diseases it is indeed a task to keep a reference book on dietetics strictly up to date. One must incorporate the newer methods which are rapidly displacing older empiric formulae; one must discriminate between recent suggestions of undoubted worth and the much more numerous unscientific dietaries still appearing in current literature; and one must use great discretion in eliminating from former editions those methods which are distinctly obsolete or inferior.

All of this the author has accomplished. While some diets now little used are retained chiefly for their historical interest, the book as a whole is thoroughly up to date. Written primarily for nurses and dietitians it should find a place on the desk of all physicians attempting to practice dietotherapy in conjunction with other medical treatment.

The first half of the book is devoted to a consideration of foods and their preparation with special emphasis placed on those foods and delicacies which are found chiefly in the sick room. The second half deals with diets used in special diseases, such as typhoid fever, diabetes, gout, obesity, deficiency dis-

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\*The Urethra and the Urethroscope: A Manual of Practical Urethroscopy. By F. Carminow Doble, M.R.C.S., L.R.C.P., London. Temporary Captain R. A. M. C. Officer in Charge Gonorrheal Division, Military Hospital, London. With fore-word by Major A. F. Frost, O.B.E.R.A.M.C. Officer Commanding Military Hospital, Rochester Row. Pp. 129. Cloth. Henry Frowde & Hodder & Stoughton, London, 1923.

†Practical Dietetics. Diet in Health and Disease. By Alida Frances Pattee. Graduate, Department of Household Arts, State Normal School, Framingham, Mass. Former Instructor in Dietetics, Bellevue Training School for Nurses, Bellevue Hospital, New York City. Former Instructor at Mount Sinai, Hahnemann, and the Flower Hospital, Training Schools for Nurses, New York City; Lakeside, St. Mary's Trinity, and Wisconsin Training Schools for Nurses, Milwaukee, Wis.; St. Joseph's Hospital, Chicago, Ill., St. Vincent de Paul Hospital, Brockville, Ontario, Canada. Fourteenth edition. Completely revised. Cloth. Pp. 687. Price \$2.60. A. F. Pattee, Publisher, Mount Vernon, New York, 1923.

eases, ulcer, nephritis, anemia, etc. The chapter devoted to postoperative diets, infant feeding and the feeding of young children should be found to be particularly useful.

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### *The Spectroscope*

THE first edition of this book came out in 1907 and many changes have been made to bring this second edition up to date. It is still a book of modest size (about 200 pages) that is intended to help the man who actually uses a spectroscope.

After a chapter on the refraction of light and on the formation of spectra the author describes the various forms of spectroscopes, their adjustment and their operation. He also adds two chapters on spectrophotography.

The difficult problem in a book of this size is to decide what to leave out. For example, he has about one page on Bioluminescence (the emission of light by living organisms) and it is doubtful if he can give enough in such a small space to make it worth while. On page 180 he refers to Planck's quantum and to Bohr's atom and here again it is doubtful if he gives enough to make such highly technical and theoretical subjects intelligible. In this connection he makes the statement, "Bohr concludes that when an electron is thrown out from one stationary motion to another it radiates out abruptly the difference of energies represented by the change in orbit. Now, apart from the difficulty of the expression "stationary motion" it is generally assumed that when the electron is "thrown out" from a stable orbit to one less stable it absorbs energy and then radiates energy when it falls back to the more stable orbit.

Typographical errors are as difficult to keep out as are flies from a well regulated home and are quite as annoying to the author. On page 103 is the statement, "Over thirty years ago Rowland photographed the solar spectrum up to 7300 A.U." This is evidently a hang-over from the first edition which failed to get revised. On page 170 is found  $10^{-18}$  instead of  $10^{-8}$  thus making the x-rays ten thousand million times too short.

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\*The Spectroscope. By T. Thorne Baker, A. M. I. E. E., F. R. P. S. Author of "Radiographic Technique." Cloth. Price \$3.00. Second edition. Pp. 208. William Wood & Company, New York, 1923.

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<sup>1</sup>Billings, J. S.: Our Medical Literature, Trans. VII Intern. Med. Congress, Lond., 1881, i, 54-70.

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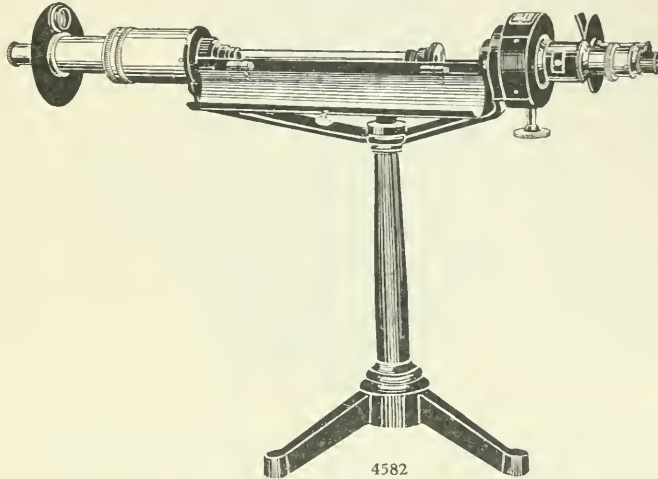
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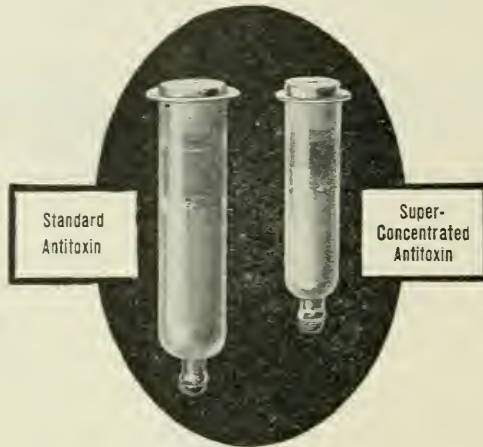
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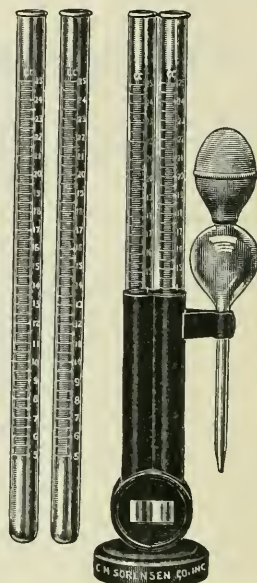
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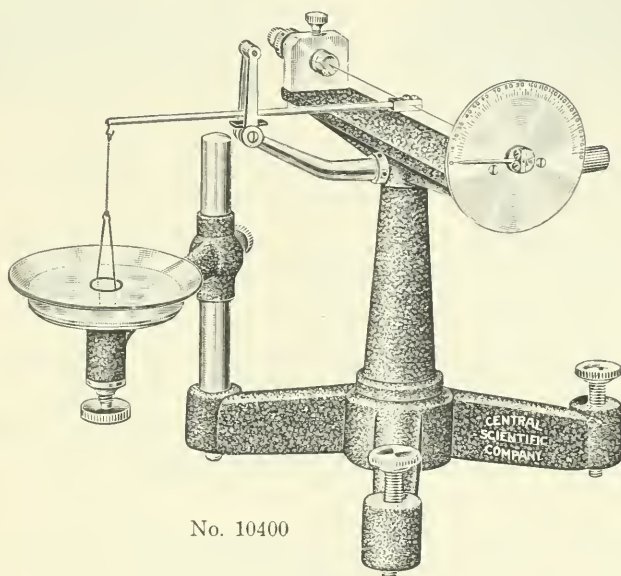
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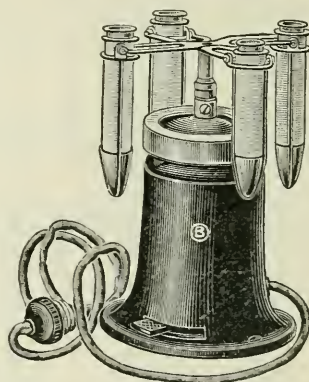
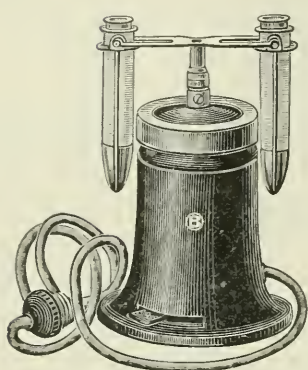
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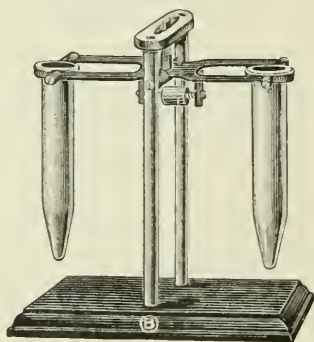
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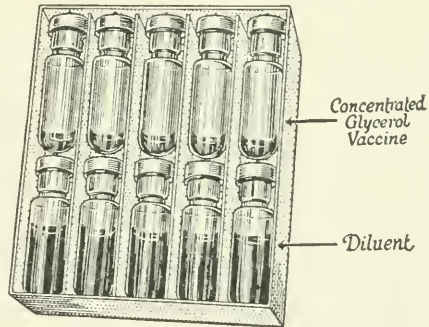
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LEDERLE ANTITOXIN LABORATORIES

511 Fifth Avenue

New York



# Directory of New and Important Laboratory Books

For the benefit of our readers we have decided to run in each issue of the Journal a list of new and reliable books that are of interest and value to the medical laboratory worker.

Our vision of the service the Journal of Laboratory and Clinical Medicine can render its readers includes its being consulted constantly for information pertaining to the best in books and equipment for the laboratory as well as for the worth-while scientific contributions in this field.

The laboratory worker advances in accordance with his own efforts and by bringing to his assistance the work of other men engaged in similar work, but without instruments of precision and truths established by other workers to help, one's progress at best is slow and laborious.

We trust that this directory of new and important laboratory books will serve to keep the many thousand readers of this Journal in intimate touch with what their confreres are doing, and in doing this render a distinct service. (See following pages for further explanation and directions in ordering.)

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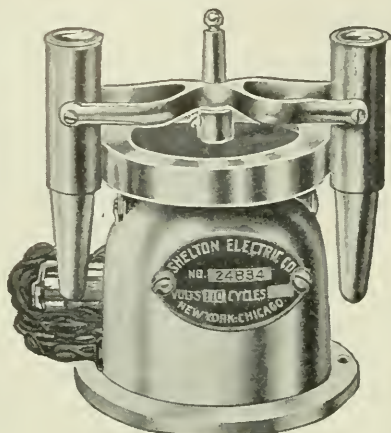
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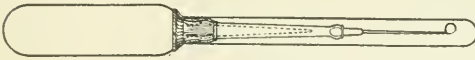
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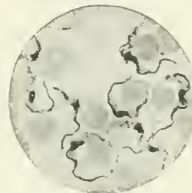
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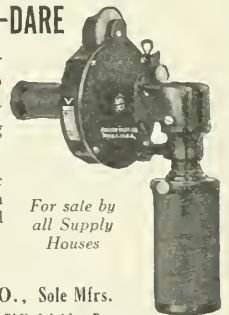
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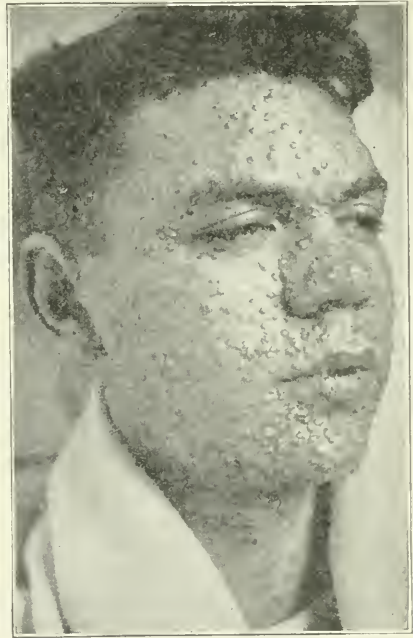
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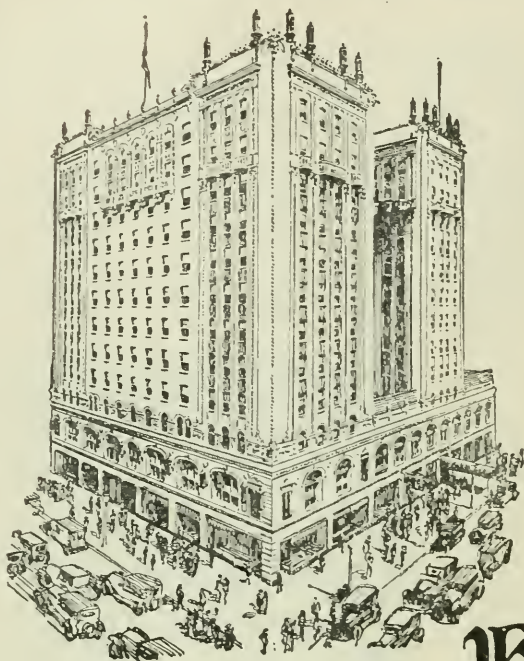
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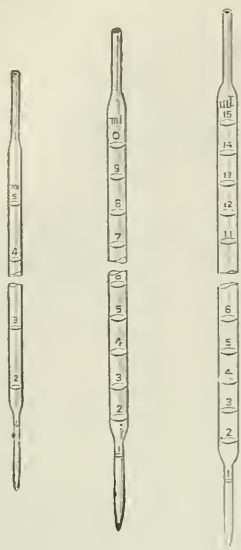
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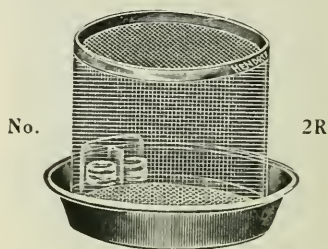
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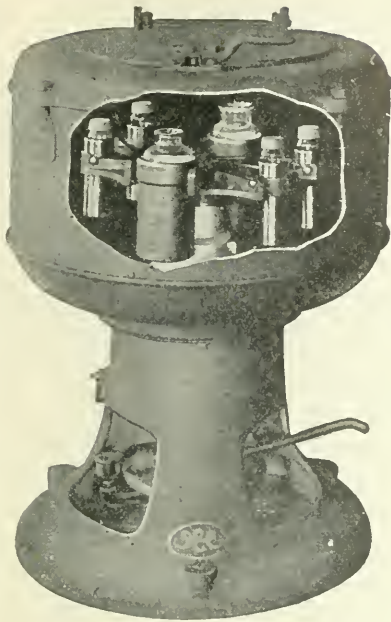
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